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OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

HEREDITY AND ORGANIC SYMMETRY IN ARMADILLO QUADRUPLETS.

I. MODES OF INHERITANCE OF BAND ANOMALIES.

H. H. NEWMAN.

INTRODUCTION.

Since the winter of 1909, when I first secured and began the study of advanced polyembryonic fetuses of the nine-banded armadillo, I have been struck not only by the striking resemblances among the individuals of a set of quadruplets but also by certain equally striking differences. It was early noted that well defined anomalous arrangements of scutes in the armor bands were sometimes repeated with closely similar detail in two or more fetuses, and were totally absent in others. Occasionally all four fetuses of a set showed a highly localized anomaly, but differed materially in the extent of the irregularity and in its symmetrical relations.

The full significance of these conditions did not dawn upon me, however, until some years later, when a set of quadruplets was obtained in which the band anomaly in question was found to be present not only in all of the four fetuses but in the mother as well. Previous to this time only a few anomalous sets had been studied and these happened to have normal mothers, a circumstance which led to the belief that these characters were not strictly inherited from parents but were merely predetermined early in embryonic life before the separation of the four embryos from the originally single embryonic vesicle. The finding of one unequivocal case of the direct inheritance from the mother of the anomalous character stimulated a new interest in the problem and made it necessary to collect a large amount of new data. Accordingly in the winter of 1911 I journeyed from Chicago to Texas and spent about a month in the armadillo country collecting material, obtaining nearly two hundred advanced polyembryonic sets together with the armor of the mothers.

A study of this material by my students and myself has brought to light a situation so interesting, but withal so intricately complex, that I almost despair of being able to render an intelligible account of it. Yet I cannot but be impressed with the uniqueness of the data and with the fact that it contains clews, afforded by no other material, as to methods of attacking some of the problems of the mechanics of hereditary transmission and of the development of organic symmetry.

In presenting the results of the study of the heredity of band and scute anomalies I have found that the problems of heredity, and that of the symmetrical distribution of these inherited characters among the quadruplet embryos, are inseparably parts of a larger problem that has to do with the mechanics of organic symmetry. When, for example, it is found that a bilateral anomaly in a mother is inherited unilaterally in the various offspring and appears in reversed symmetrical relations in twins, we have something more than a case of simple inheritance. When again we find a unilateral anomaly in one pair appearing bilaterally without reversed symmetry in the opposite pair we see the problem in a more complex form. When, still further, we note that an anomaly of a single scute in the mother is inherited as such in some of the offspring and as a whole series of anomalous scutes (a band anomaly) in others, we begin to suspect that the problem is too intricate for any simple solution and that only in certain of its aspects is it likely to yield to analysis. Yet it is our duty to carry the analysis as far as our data allows.

That the circumstance of polyembryony vastly complicates the already sufficiently complex problems of heredity can scarcely be denied, but it is confidently hoped that the new relations introduced by this unique type of development may throw light from a different angle upon certain phases of heredity that are now quite obscure.

Before the reader can appreciate the significance of the data on heredity and organic symmetry, it will be necessary for him to be made familiar with the character of the material and its specific frequency and distribution.

MATERIAL AND METHODS OF STUDY AND PRESENTATION.

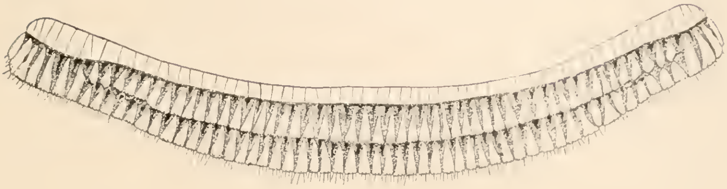
The material for this investigation consists of nearly two hundred sets of quadruplet fetuses in advanced stages, showing the definitive arrangement and number of scutes in the armor. In every case the shell of the mother was obtained and preserved so that there is on hand complete data on uniparental inheritance of armor characters. The fetuses and the respective mothers make up a collection of nearly one thousand individuals, which is unquestionably a representative sample of the species.

Counts of scutes and records of anomalies have been made independently by at least two workers and all differences or discrepancies have thus been checked and corrected, so that the probability of error and personal bias have been eliminated.

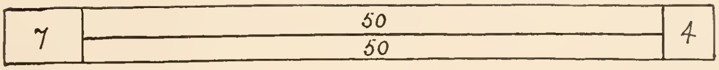
Since the anomalies here dealt with are rare, occurring in only about three per cent. of individuals, I have made an examination of the preserved armor of 1,800 adults that formed the stock of a single dealer. This was done in order to determine the limits of diversity and the specific distribution of the anomalies. A complete record of all these anomalies has been made in the form of a pictorial diagram in Table *A*, 1, 2 and 3.

In order that the reader may understand the nature of the anomalies it will be necessary to give a brief statement of the normal relations of armor units. The primary unit of the armor is a threefold complex consisting of a bony plate, covered by a primary epidermal scale of scute, and a group of hairs that are imbedded in the bony plate and perforate the scute. For convenience this complex is called a "scute." In young fetuses the outline of the epidermal scute is all that can be seen; but this visible unit stands for the whole complex. These scutes or armor units of the carapace are arranged differently in the three main subdivisions of the armor, the pectoral, banded and pelvic shields. In the pectoral and pelvic regions the bony plates are arranged like tiles and form a rigid immovable shield. Naturally the primary scutes are arranged in more or less regular transverse rows, but these rows seldom run straight across the shield. Instead it is commoner for a row that starts single on the margin to become double for some distance where the bulge of the shell is greatest, then to be single again toward the middle. Again a row may start double laterally and be single for a short distance toward the middle. Irregularities in scute rows are commonest near the posterior border of the pectoral shield next to the banded region.

In the banded region the arrangement of scutes is quite different. The bony plates are elongated antero-posteriorly to form rows of units, in general appearance somewhat like the keyboard of a piano. The posterior margin of the first band is free from and slips over the anterior margin of the second, an arrangement that lends flexibility to the armor. The second band bears a similar relation to the third and so throughout the nine movable bands. Each bony plate is accompanied by and partially covered by an elongated, wedge-shaped primary scute. Figs. 1 and 3 represent two bands removed whole from the armor. The typical band, however, is composed of an even array of scutes arranged in a single row from margin to margin. Only one third of one per cent. of the 16,200 bands examined are in any way irregular in the arrangement of the armor units. The rare exceptions, however, are of especial interest and constitute the anomalies that are the subject of the present study. These peculiar bands belong for the most part to the types, the details of which are shown in Figs. 1, 3 and 5. They consist of bands that are partly single and partly double. In Fig. 1 is seen a very common type of bilateral anomaly in which a few scutes on each margin constitute the single part of the band, while the main central region is double. In Fig. 3 we see an equally common condition in which the double part of the band is confined to a number of scutes starting at some distance from the margins but stopping short of the middle portions of the band. Similar conditions of greater or less extent are found unilaterally as frequently as bilaterally. Now these irregular bands are in no sense abnormalities or products of injury, but are of the same nature exactly as are the irregulari-



1



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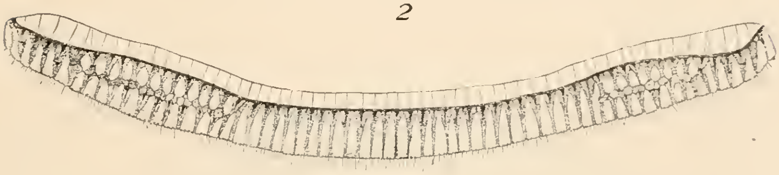

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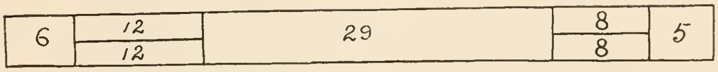
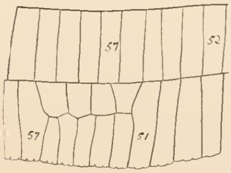

6
12
12
29
8
8
5

4



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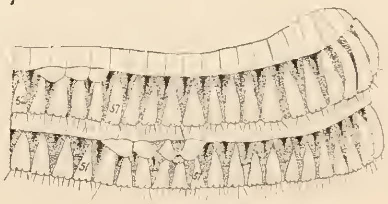

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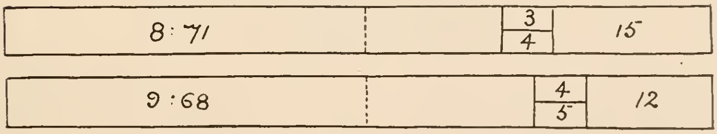
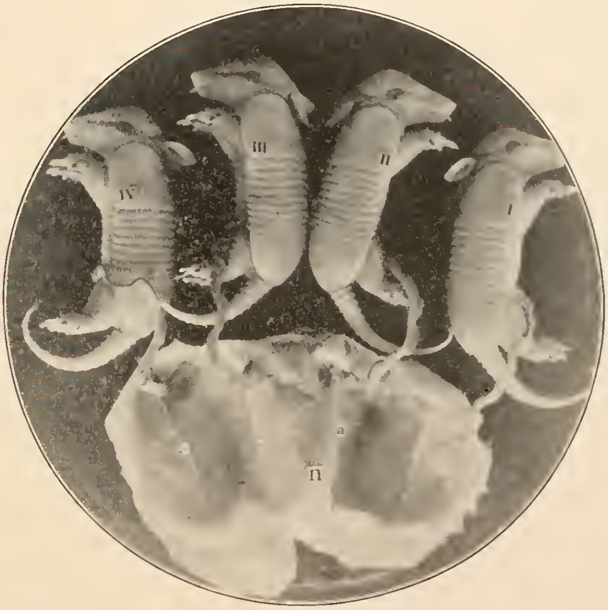

8 : 71
3
4
15
9 : 68
4
5
12

7

ties of scute rows in the adjacent pectoral shield, where regularity is rare and irregularity the typical condition. In about three per cent. of individuals the irregular conditions typical for the posterior part of the pectoral shield invade the adjacent parts of the banded region. That this is the correct interpretation of the anomalies in the banded region is evidenced by the fact that out of 84 band anomalies recorded for this study 73 occur in the first band, which is contiguous to the pectoral shield. Of the remaining eleven cases 7 occur in band 2, 1 in band 3 and 1 in band 8, which is near the other irregular region, viz., the pelvic shield. Irregularities never occur in the middle bands of the carapace, which are farthest from the irregular pectoral and pelvic shields. Another way of interpreting the facts is to look upon the arrangement of scutes in the banded region as due to mechanical adjustments of the primordia of armor elements to flexures during embryonic development. Typically the banding confines itself to the abdominal parts of the armor but occasionally comes in a little farther forward or a little farther back and thus includes parts of the pectoral and pelvic carapace with the typical peculiarities of scute arrangements of these regions. As a result we have these anomalous cases in which the first or second band has the characteristics, doubling, etc., of the pectoral region, and the posterior bands have similarly the characteristics of the pelvic region.

An irregularity in a given band of the banded region may involve as few as two or even one scute. Such an irregularity may consist of a single scute or two in an otherwise double band or a double scute or two in a band otherwise single. The latter situation is much the commonest of all anomalies studied. Sometimes the anomaly manifests itself by a more or less complete longitudinal or diagonal splitting of a single acute. Such conditions are to be dealt with separately as there is a considerable mass of interesting data on the inheritance and distribution of these anomalous double scutes. It is impossible, however, to deal with the inheritance of anomalous band conditions without discovering the intimate genetic connection that exists between band and scute anomalies, for sometimes a scute anomaly in a parent reappears as a band anomaly in offspring and vice versa.

After many experiments in tabulating the occurrence of band and scute anomalies I have adopted a simple pictorial scheme which will readily explain itself on examination of the diagrammatic Figs. 2, 4 and 7 which are simplified representations of the scute conditions shown in Figs. 1, 3 and 5 respectively. The numbers of scutes in both double and single regions are represented by arabic numerals and the number of the band is indicated sometimes, as in Table *A*, 1, 2 and 3, by the abbreviation Bd. 1 or Bd. 2 just above the margin of each band, and sometimes as in table B by a single arabic numeral followed by a colon and the total number of scutes in the band. When the quadruplet fetuses and the mother are dealt with, the number of the set is indicated as A.101 ♀ or K.40♂, indicating at the same time the sex of the litter; the bands of the mother are labeled M and those of the fetuses I., II., III. and IV. When the mother or any fetus is not tabulated the inference is that no anomaly is present in the omitted individuals. Other schemes of tabulation, such as the circular figures and those showing double scutes will be explained in the proper place.



Specific Distribution and Frequency of Band Anomalies.

Little need be added to the data given in Table *A*, 1, 2, 3, which shows the anomalies found in 1,800 adult specimens. On previous occasions I have made records of over 1,000 other specimens and have failed to find any other types of diversity than those shown in this table. So it may be considered as established that we have before us in this collection an adequate representation of the diversity of band anomalies and their distribution among the various bands. Although no two irregular bands in unrelated individuals are just alike there are certain well-defined classes of anomaly such as the bilateral (symmetrical or asymmetrical) and the unilateral. There are types single on the margins and double in the middle; there are types double at the margin and single in the middle; and there are mixed types.

Specimens 1–14 show various types of anomaly in which the bands are single at the margin with bilateral regions of doubling. Specimens 15 to 35 show unilateral expressions of the same types of anomaly. Specimens 36–42 show various mixed types, which are perhaps reversals of anomalies of the two sides of the individuals. Fig. 42 is an especially interesting case of such a reversal of symmetry, for the right and left halves of the band are duplicates but are not mirror-image effects. They bear the same relation to each other as the reversed finger prints found occasionally on the right and left index fingers of human duplicate twins, as shown by Wilder.

Some of the cases of exact bilateral symmetry, such as those in specimens 5, 7 and 36 are very significant, and there are all degrees of inexact bilateral duplication of anomalies ranging from specimens 12, 4, 8, 11, 14 down to specimens 13, 10, 9, 6, etc.

It must not be forgotten that there are no two anomalies alike in nearly three thousand specimens taken at random. When, therefore, we find, as we soon shall, exact duplicate anomalies in two or more fetuses in a set we shall not be able to explain them as coincidences.

Having made clear the nature of the anomalies and their diversity and distribution in the species, we are now in a position to examine the data on the inheritance of these characters.

TABLE A1.

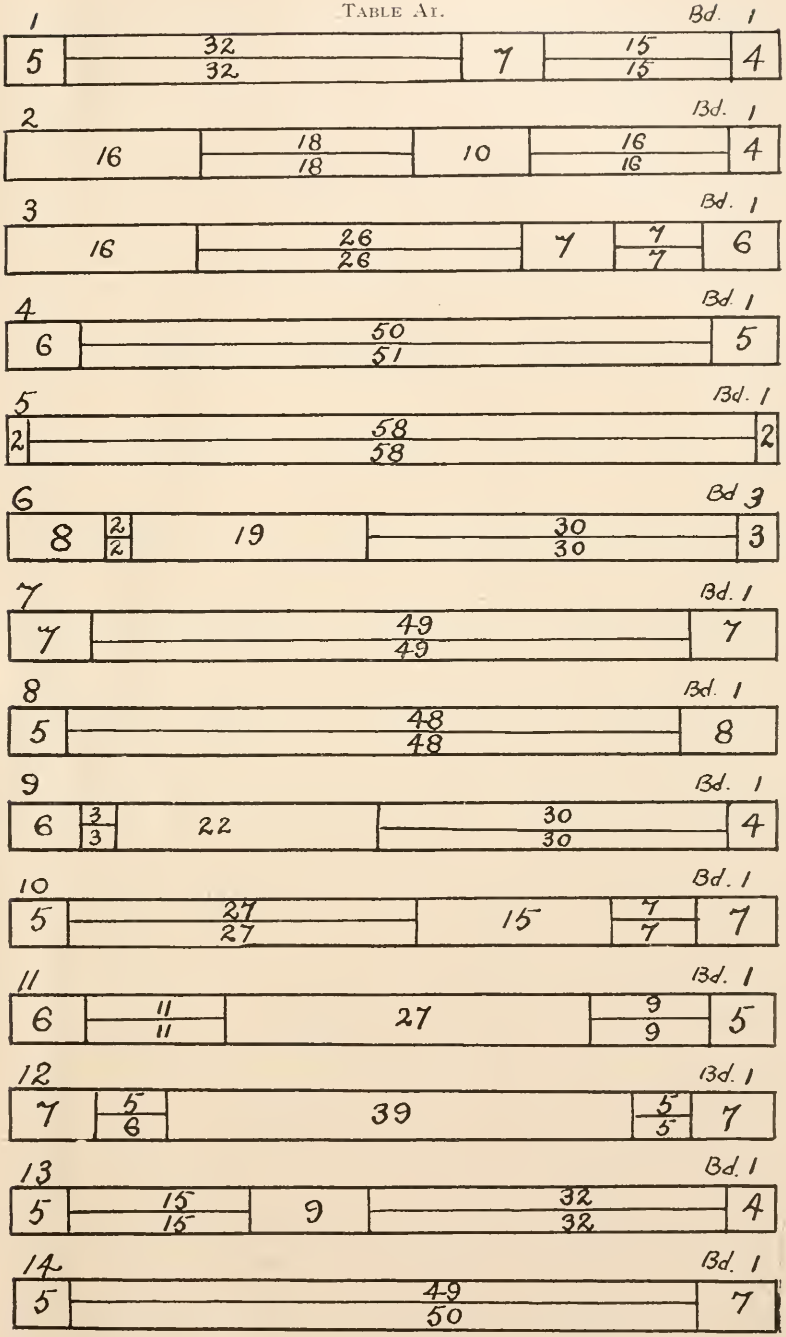

Bd. 1
Bd. 1
Bd. 1
Bd. 1
Bd. 1
Bd 3
Bd. 1
Bd. 1
Bd. 1
Bd. 1
Bd. 1
Bd. 1
Bd. 1
Bd. 1

TABLE A2.

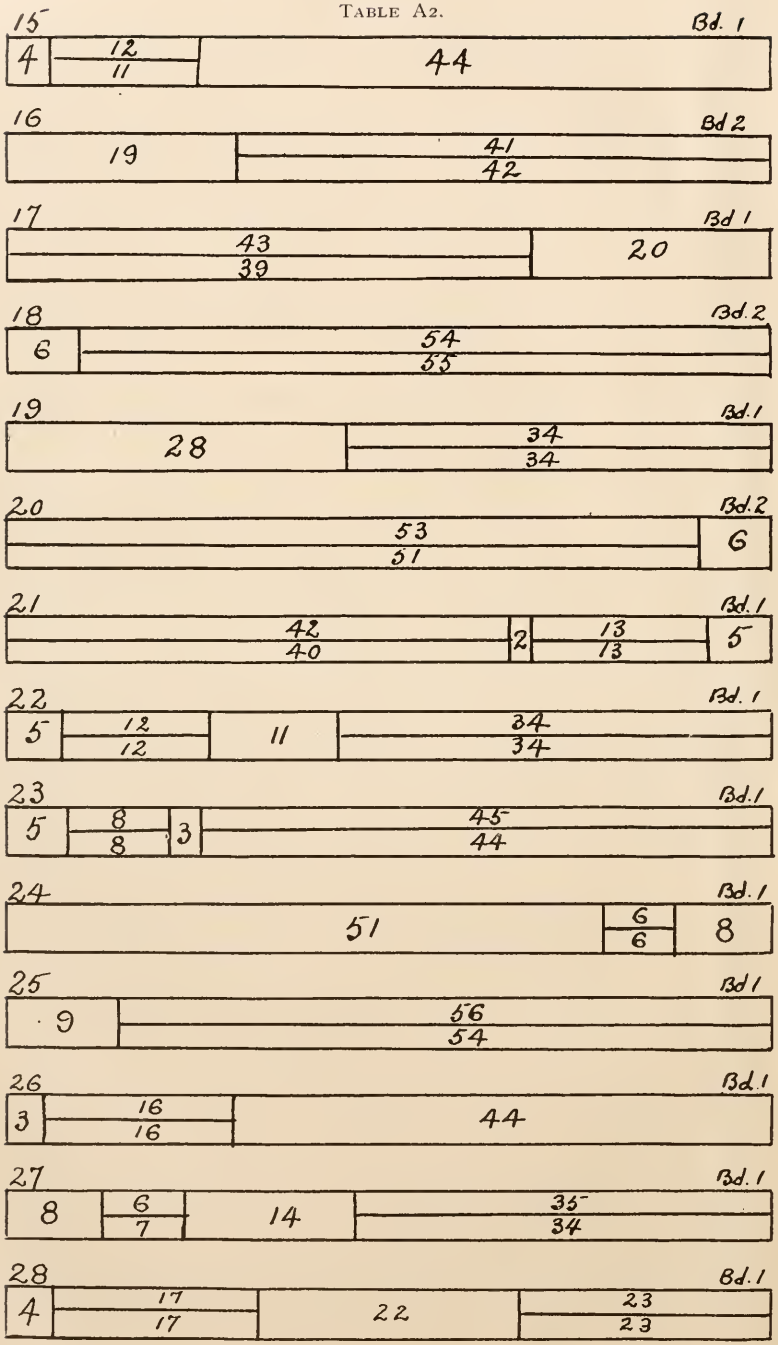
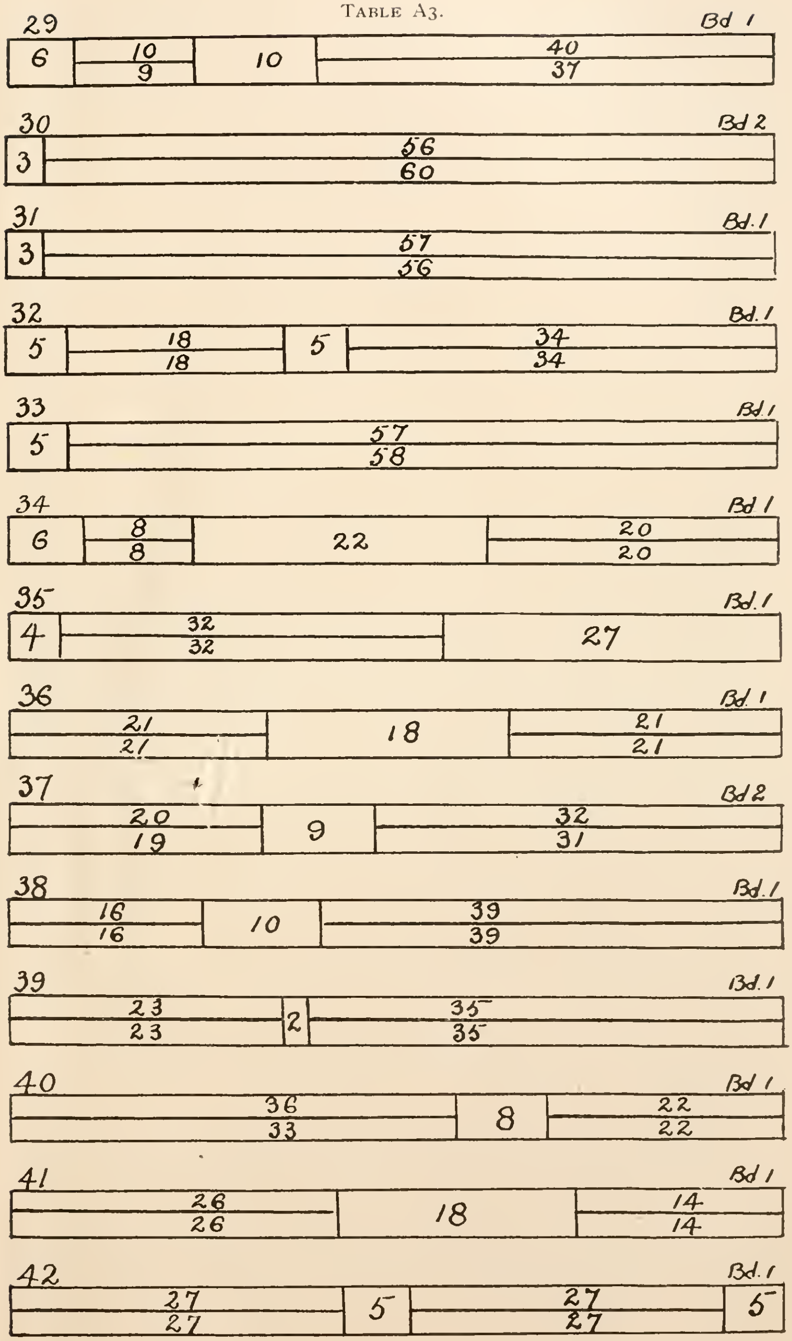

Bd. 1
Bd 2
Bd 1
Bd. 2
Bd. 1
Bd. 2
Bd. 1
Bd. 1
Bd. 1
Bd. 1
Bd 1
Bd. 1
Bd. 1
Bd. 1

TABLE A3.


Bd 1
Bd 2
Bd. 1
Bd. 1
Bd. 1
Bd 1
Bd. 1
Bd. 1
Bd 2
Bd. 1
Bd. 1
Bd 1
Bd 1
Bd. 1

THE INHERITANCE AND DISTRIBUTION OF BAND ANOMALIES.

After a study of over 150 of the most advanced sets of quadruplets in my collection I am convinced that both band and scute anomalies are strongly inherited. In every case in which a mother exhibits a band or a scute anomaly, a related anomaly is found in one or more of the offspring. If the character were not inherited as a dominant, one would expect some exceptions to this rule, but none have been found. When, therefore, we find an equal number of offspring of normal mothers exhibiting anomalies of the same sort we are justified in concluding that the characters represent a heritage from the unknown fathers. This assumption is farther justified by the finding that the characters in question are neither sex-limited nor sex-linked.

For our purposes, then, the data here published are adequate in that a study of uniparental inheritance reveals fully the modes of inheritance that obtain for band and scute anomalies. Whatever genetic relations are found to hold between mothers and offspring would doubtless hold for fathers and offspring. The only unfortunate complication that is encountered is in connection with a small per cent. of cases in which both fathers and mothers possess anomalies. A few sets of fetuses are obviously of this dual anomalous parentage, and we can, by knowing the maternal anomaly, make a well-founded conjecture as to the probable nature of the anomaly in the unknown father.

Whether or not I am justified in assuming that the study of maternal inheritance reveals the essential facts concerning the inheritance of the characters in question, can be settled only by breeding and, as has been pointed out in extenso in an earlier paper (Newman, '13), breeding experiments with the armadillo are at present totally impracticable. Consequently, we are forced to rely upon a study of inheritance from one parent, the mother.

The nature of the anomalies is such that I have been unable to devise any really convenient method for tabulating the facts that must be known about them. It seems necessary to consider each case of inheritance separately, and this may be done without undue prolixity because the cases are not numerous. The pictorial method appears to be well adapted for the data, but it would be too tedious a task to draw each anomaly in detail after the fashion of Figs. 1 and 3. Instead I shall use the diagrammatic form seen in Figs. 2 and 4, which give all the necessary information. The best method I have been able to devise for showing the symmetrical or asymmetrical relations of the anomalies and their distribution among the quadruplet fetuses of a set is that shown in Figs. 9–16, in which the anomalous bands are placed as though within the embryonic vesicle. The reader must remember that the structures studied are integumentary units, that the embryonic vesicle is so inverted that the ectoderm forms the inner lining and the endoderm is on the exterior.

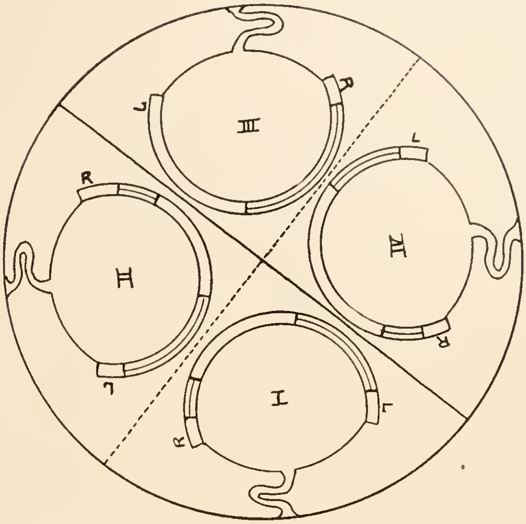
The diagram (Fig. 8) shows the relative positions of the band anomalies of the fetuses in set 87 (Fig. 9). Here we have an equatorial cross section of an advanced vesicle with the body of each fetus severed at the first armor band where the anomaly occurs. The ventral surfaces face outward and are attached by their respective umbilical cords to the placental portion of the vesicle. The dorsal aspects are turned inward. Right and left sides of each fetus are indicated. The reader must imagine himself in the center of the vesicle facing outward toward each fetus. In order to simplify this type of diagram, I have found it best to ignore all structures but the band in question and to represent the latter as though straightened out against the periphery. The reader will understand the adopted form of diagram after a comparison of Fig. 8, which represents nearly the actual relations present in set 87, and Fig. 9, which is a diagram of the same set showing only the bands that are of interest in this study. In both figures the solid quadrant line divides the twins I. and II. from twins III. and IV. and the dotted line separates twin individuals, I. from II. and III. from IV. In the actual vesicle these quadrant lines are occupied by amniotic partitions that hermetically isolate each fetus from the others. When the anomaly is found in the mother also the material condition is indicated in a straight band diagram beside or beneath the circle showing the offspring. When any individual shows an anomaly in more than one band, the more anterior anomaly is shown in the circle and the more posterior anomaly in a concentric sector outside of the circle, as in Fig. 9.

Of the twenty-six sets of fetuses one or more individuals of each of which show band anomalies, twelve came from mothers that also showed either band or acute anomalies, and fourteen came from mothers that showed no anomaly. Presumably the anomaly has been inherited from the father in these fourteen cases.

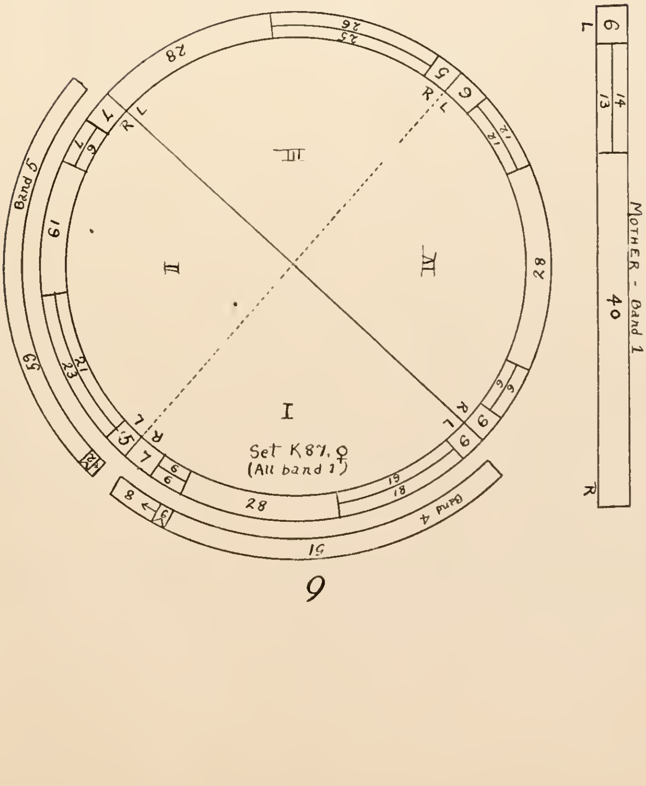
Of the twenty-six sets showing band anomalies exactly half are male and half female, which demonstrates that the characters dealt with have no sex-limitation or sex-linkage. Males and females inherit equally strongly from the mothers, for of the twelve sets of offspring in which the mother also shows an anomaly, five are of the female and seven of the male sex. In the case of four sets with anomalous mothers and similarly in four sets with normal mothers, I shall follow the plan of placing a circular diagram and the verbal description and analyses close together in the text. The remaining sets are tabulated in somewhat more compact form and placed at the end of the paper. Those in Table *B* are the eight remaining sets that have the anomaly in both mother and one or more offspring; those in Table *C* are the remaining ten sets with band anomaly in one or more fetuses but with normal mothers. Each set is placed in a separate block and labeled at the upper left-hand corner, with the number of the set and the sex of the young, as *C* 29 ♂. The number of the band showing the anomaly is indicated in two ways, as bd. 1 or bd. 2 placed next to or on the band, or by a number placed on the band followed by a colon and the total number of scutes in the band, as 7: 65, meaning band 7 having a total of 65 scutes. In bands more or less broken by doubling the number of scutes in each part is indicated. In the case of short series of double scutes or one double scute, the number placed on the element, together with the arrow, indicates the numerical position of the element from either margin, or from the middle, which is indicated by a dotted line.

Set K. 87, ♀ (*Figs. 8 and 9*).

This set shows more certainly than any other the direct inheritance of a material band anomaly by all of the offspring. In the mother the anomaly consists of a unilateral local doubling


III
L
R
IV
L
R
II
R
L
I
L
R

8


R
L
III
R
L
Band 5
II
IV
L
R
I
R
L
Set K87, ♀
(All band 1)
8 ←
Band 4
L
Mother - Band 1
R

9

of band 1, confined to the left half of the band and almost identical with the left half of the band pictured in Fig. 3. The band begins on the left lateral margin with six single scutes, is then double for 14–13 scutes and single for the remainder of the series. No other anomalies are exhibited by the mother. Fetuses I., II. and IV. show similar anomalies of band 1, but they are unequally bilateral in their expression (like Fig. 3), all three showing more scutes double on the left than on the right. Fetuses I. and II. are strikingly similar in the distribution of single and double scutes and show no reversal of symmetry. Fetus IV. is the most nearly bilaterally symmetrical in the disposition of its scutes. Fetus III. is unilateral like the mother, but shows a reversal of symmetry in that the doubling is confined to the right side. The symmetry of fetus III. is also a reversal of that of fetuses I., II. and IV., which show a preponderance of doubling on the left side. In addition to the anomalies referred to, fetuses I. and II. show scute anomalies of bands 4 and 5 respectively that do not appear to be traceable to the mother and have presumably come from the father. This is one of the rare sets in which it is probable that anomalies came from both sides of the family. The double scutes of I. and II. are in a reversed symmetry to each other, being near the right in fetus I. and near the left in fetus II.

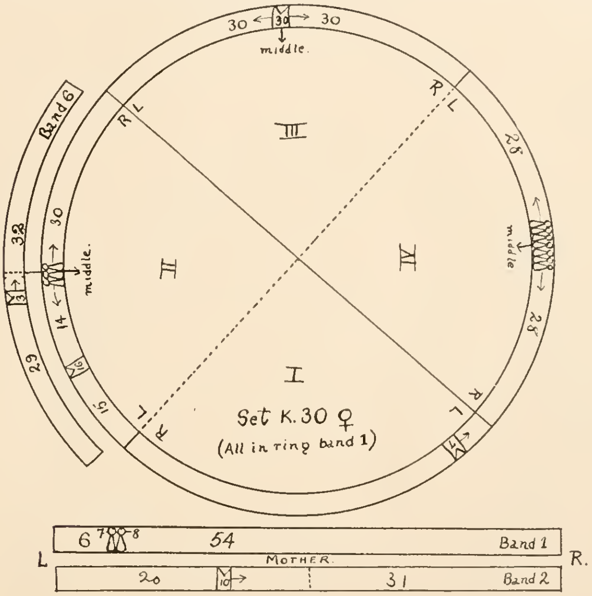
Set K. 30, ♀ *(Fig. 10).*

This set is of importance in that it deals with short rows of double scutes ranging from 2 to 7 elements and serves to emphasize the genetic connection between plate and scute anomalies.

The mother has in band 1 in the positions 7 and 8 from the left two adjacent double scutes forming a band anomaly of minimal size but of the same character as the short double regions of the band pictured in Fig. 5. If only one scute had been double-tiered, we would have called it a "scute" anomaly instead of a "band" anomaly. There is also in the mother an independent double scute situated 10 places to the left of the middle of band 2.

Fetus I. has a double scute in band 1 in exactly the position of the first of the two double scutes of the mother. This seems to indicate that a double scute and an incipient double band are genetically equivalent.

Fetus II. has in the exact middle of band 1 a short series of three double scutes like the two which are near the left margin of the mother; and in the same band, midway between the middle and left band margin, one double scute. In band 6 of the same fetus there occurs at a point three scutes to the left of the middle another double scute like that in band 1. Note that all asymmetrical anomalies are on the left side as in the mother.



10

Fetus III. has one double scute in the exact middle of band 1, or in a position identical with the short double band in fetus II.

Fetus IV. has a double region of 7–7 scutes in the exact middle of band 1. This doubling is simply a more pronounced case of the doubling shown in the mother as in fetuses II. and III.

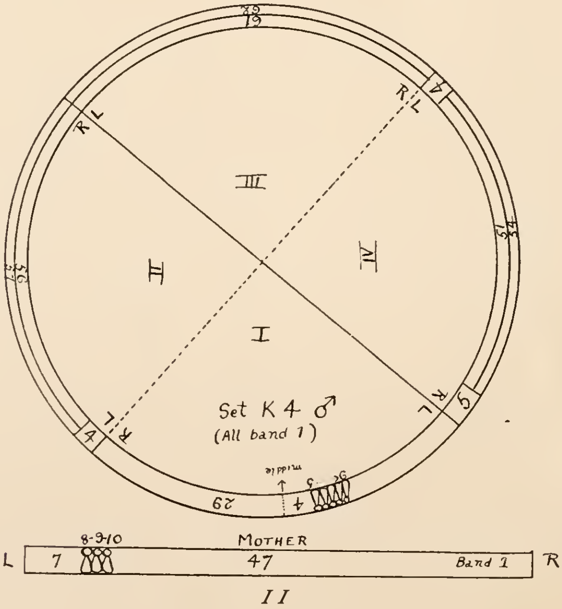
The points of interest illustrated by this set are:

1. In mother and offspring the unilateral symmetry is the same throughout except that there is a tendency for the anomaly to shift to the center of the band. This shifting of lateral anomalies to the center is a peculiar type of symmetry reversal in which the central and lateral portions of each half band become

reversed. That this is the correct interpretation of the situation is amply shown by many cases shown subsequently.

2. There occur several examples of the reduplication of a single inherited unit, sometimes involving a single band, sometimes two or more. Doubtless the anomalies of band 1 and band 2 in the mother represent a merely reversed reduplication of the same inheritance unit. Similarly in fetus II. the series of three double scutes in the middle of band 1, the double scute near the middle of band 6 and the scute in the middle of the left half of band 1 are varied manifestations of the same anomaly.

3. There is more extensive doubling in the two primary bud individuals II. and IV. than in the two secondary bud individuals I. and III.



Set K. 4, ♂ (Fig. 11).

The mother has a short series of 3 double scutes in positions 8, 9 and 10 from the left.

Fetus I. has a series of 5 double scutes occupying positions 5, 6, 7, 8 and 9 from the middle of the band.

Fetus II. has on the left 4 single scutes, and the rest of the band, beginning in position 5, is double. The symmetry is that of the mother, but the doubling involves most of the band. The symmetry is a half-band reversal of that in fetus I. in that the doubling begins 5 scutes from the left margin instead of 5 scutes from the middle.

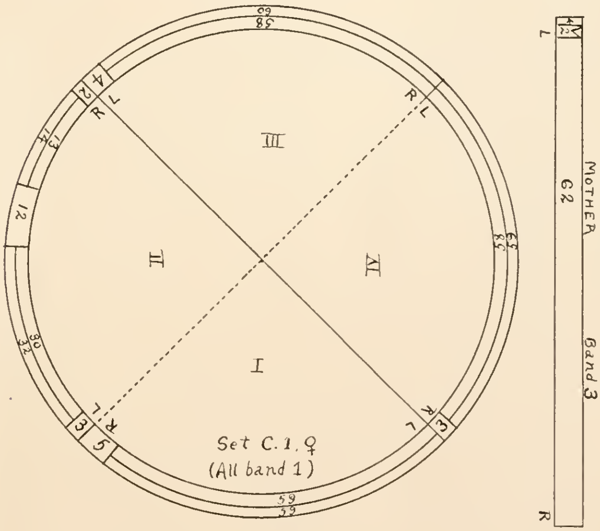
Fetus III. has either no anomaly at all or else has the entire band doubled. It is difficult to decide as to these alternatives.

Fetus IV. has the anomaly bilaterally symmetrically. One might claim that the anomaly that should have appeared on fetus III. has been transferred to fetus IV. so that the latter has a double dose of it.

Set C. 1, ♀ (Fig. 12).

The mother has in band 3 in the second position from the left a double scute.

Fetus I. has an extensive doubling beginning 5 scutes from the right-hand margin of band 1. Thus the symmetry of the mother is reversed.



12

Fetus II. has an extensive unequal bilateral doubling beginning after two scutes on the right and three on the left, continuing to the middle on the left and for 13–14 scutes on the right.

Fetus III. has the doubling of band 1, beginning 4 scutes from the left. The symmetry is the same as that of the mother, but a reversal of that of the opposite individual, fetus I. Fetus III. is therefore a mirror-image of fetus I.

Fetus IV. has the anomaly beginning after 3 single scutes from the right margin of band 1. The symmetry is a reversal of that seen in its twin partner, fetus III., and of that of the mother, so that III. and IV. are mirror-images of each other.

This set shows clearly that a double scute is genetically equivalent to a double band. It is seldom that one finds a set showing such extensive reversals of symmetry.

Set C. 29, ♂ *(Table B1).*

This set is the complement of set C. 1, in that the mother has an extensive band doubling and the offspring inherit it in the form of scute doubling.

The mother has in band 1 beginning 4 scutes from the left a doubling extending to the right margin.

Fetus II. has in band 7 the second scute from the right double.

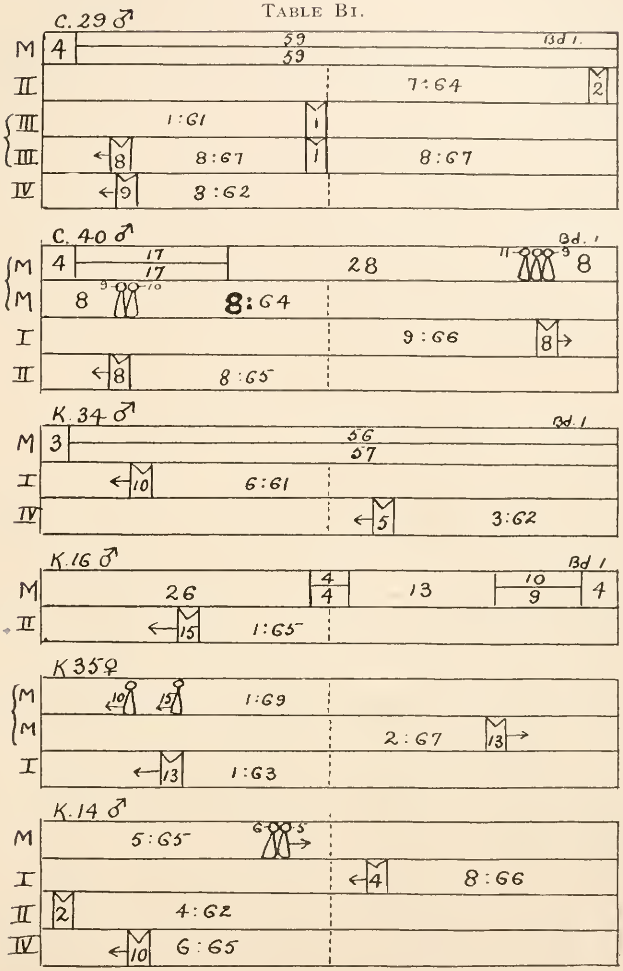
Fetus III. has in band 1 and in band 8 just to the left of the middle a double scute, and in addition a double scute 8 scutes from the left in band 8.

Fetus IV. has in band 3 a double scute in the ninth position from the left.

This set illustrates an extreme case of positional shifting of a genetic character. There can be no question of the relationship between the two scutes of fetus III., occurring in band 1 and band 8 just to the left of the middle. This is a case of reduplication of inheritance unit along the antero-posterior axis. Similarly scute 8 in band 8 of fetus III. and scute 9 in band 3 of fetus IV., are expressions at different levels of the antero-posterian axis of the same genetic unit.

The double element next to the right-hand margin of band 7 in fetus II. is certainly the same genetic unit as the element next to the middle of band 8 of fetus III., but with a reversal of symmetry. Thus by the application of the principle of symmetry reversals and reduplications down the primary axis we can connect up even such apparently unrelated units as these.

TABLE B1.


C. 29 ♂
M
II
III
III
IV
4
59
59
Bd 1.
7:64
2
1:61
1
8
8:67
1
8:67
9
8:62
C. 40 ♂
Bd. 1
M
M
I
II
4
17
17
28
11
9
8
8
9
10
8:64
9:66
8
8
8:65
K. 34 ♂
Bd. 1
M
I
IV
3
56
57
10
6:61
5
3:62
K. 16 ♂
Bd 1
M
II
26
4
4
13
10
9
4
15
1:65
K. 35 ♀
M
M
I
10
15
1:69
2:67
13
13
1:63
K. 14 ♂
M
I
II
IV
5:65
6
5
4
8:66
2
4:62
10
6:65

Set C. 40 ♂ (Table B1.)

Here the same type of inheritance is seen as in set C. 29, but the genetic relations are much clearer, and involve no strain upon one's credulity.

The mother has in band 1 a bilateral doubling, beginning on the left after four single scutes and extending in a series of 17-17 double scutes and beginning on the right after 8 single scutes and continuing for a short series of 3-3 double scutes. In addition we find in band 8 a doubling strikingly like that of the right side of band 1, but reversed to the left and involving only 2 scutes. This is another case of reduplication in anterior and posterior parts of the banded region, but there is also in this case a symmetric reversal.

Fetus I. has in band 9 in position 8 from the right a double scute. This is evidently the genetic equivalent of the two double scutes in band 8 of the mother, but the symmetry is reversed.

Fetus II. has in band 8, 8 scutes from the right, a double scute which is doubtless the reversal or mirror-image of that in its twin partner, fetus I.

Set K. 34, ♂ *(Table B1).*

In this set the genetic relations are quite obscure.

The mother has in band 1, beginning after 3 single scutes on the left a doubling of the rest of the band.

Fetus I. has in position 10 from the left margin of band 6 a double scute.

Fetus IV. has in position 5 to the right of the middle of band 3 a double scute.

Set K. 16 ♂ *(Table B1).*

The mother shows a right lateral and a median doubling of band 1.

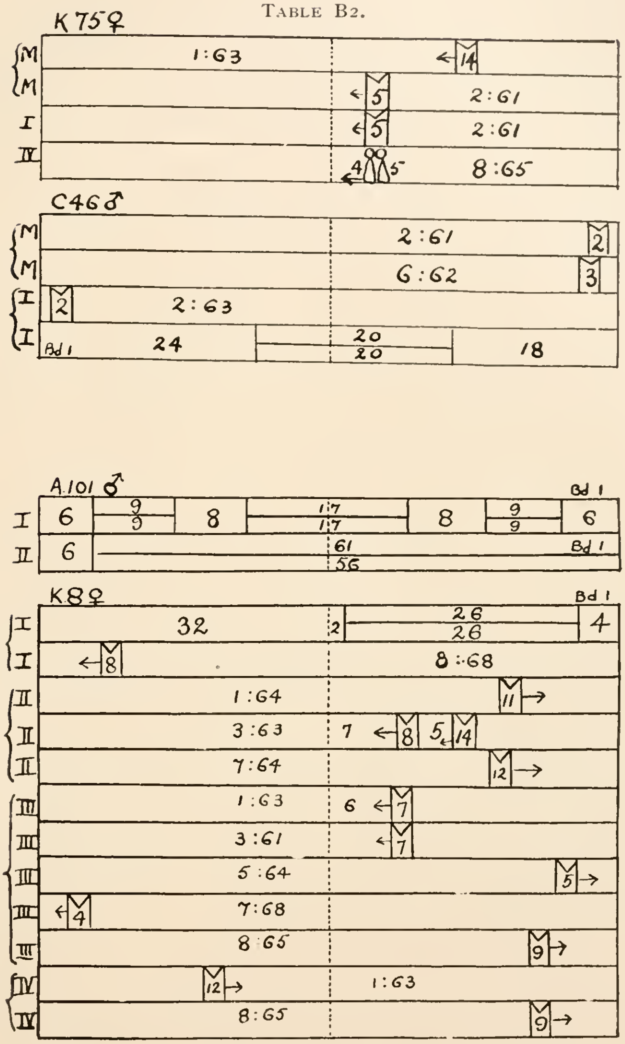
Fetus II. has one double scute in band 1, situated in the middle of the left side of band 1, another case of centro-lateral symmetry reversal.

Set K. 35, ♀ *(Table B1).*

The mother has a rather unusual anomaly in the form of two "split" scutes separated by several single scutes, occurring in band 1, in places 10 and 15 respectively from the left-hand margin. In band 2 we find the bilateral equivalent of these elements in the form of one double scute situated in position 13 from the right.

Fetus I. is the only one of the offspring to inherit the maternal anomaly. There is in band 1 a double scute situated 13 places from the left in the reverse position of that seen in band 2 of the mother. The genetic relations here are quite clear, but why should only one out of four offspring show an inherited character?

Table B2.


K 75 ♀
M
M
I
IV
1:63
14
5
2:61
5
2:61
4
5
8:65
C46 ♂
M
M
I
I
2:61
2
6:62
3
2
2:63
Bd 1
24
20
20
18
A.101 ♂
Bd 1
I
II
6
9
9
8
1:7
1:7
8
9
9
6
6
61
56
Bd 1
K8 ♀
Bd 1
I
I
II
II
II
III
III
III
III
III
IV
IV
32
2
26
26
4
8
8:68
1:64
11
3:63
7
8
5
14
7:64
12
1:63
6
7
3:61
7
5:64
5
4
7:68
8:65
9
12
1:63
8:65
9

Set K. 14 ♂ (Table B1).

The mother has an incipient double band of two scutes in positions 5 and 6 to the left of the middle of band 5.

Fetus I. has a double scute in position 4 to the right of the middle of band 8. This shows reversed symmetry and a shifting down the primary axis of the genetic unit inherited from the mother.

Fetus II. has a double scute in position 2 from the left-hand margin of band 3. This is evidently a reversal of the conditions seen in the mother and is also a complex reversal, with shifted position on the primary axis, of the condition seen in its twin partner, fetus I.

Fetus IV. has a double scute in band 6, situated with reference to the middle of the left half band in about the same relation as is the peculiarity of the mother with reference to the middle of the whole band. In other words it is 5 scutes to the left of the middle of the left half of band 6, while that of the mother is situated 5 scutes to the left of the middle of band 5. Considering the rarity of anomalies of any sort in the bands 3, 4, 5 and 6, there can be no doubt of the genetic identity of these elements. Granting this the interpretation offered is not forced.

Set K. 75, ♀ (*Table B2*).

Here the mother has two double scutes, one near the middle of the right half of band 1 and another to the right of the middle of band 2.

Fetus I. has a double scute in band 2 identical in position with one of those of the mother.

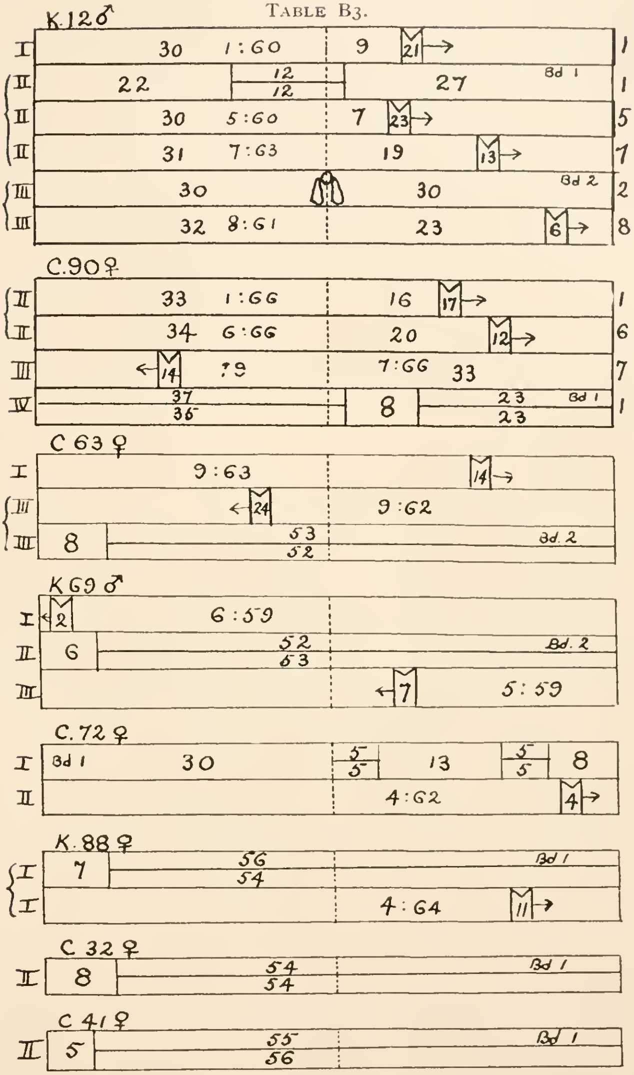
Fetus IV. has in band 8, in a position with reference to the middle identical with one of those of the mother and that of fetus I., an incipient band doubling of 2 scutes. This is another example of the shifting of an anterior element to a position further down the primary axis. It also emphasizes the genetic identity of band and scute doublings.

Set C. 46, ♂ (*Table B2*).

The mother shows a reduplicated double scute anomaly in bands 2 and 6 in positions 2 and 3 respectively from the right-hand margin.

Fetus I. shows in band 2 an exactly reversed mirror-image reversal of the anomaly in band 2 of the mother and in addition an extensive doubling in the middle of band 1. This illustrates three points; (*a*) reduplication down the primary axis, (*b*) the genetic relation between scute and band doubling, (*c*) the half-band type of reversal of symmetry.

TABLE B3.


K.12♂
I 30 1:60 9 21 1
II 22 12 12 27 Bd 1 1
II 30 5:60 7 23 5
II 31 7:63 19 13 7
III 30 30 Bd 2 2
III 32 8:61 23 6 8
C.90♀
II 33 1:66 16 17 1
II 34 6:66 20 12 6
III 14 79 7:66 33 7
IV 37 36 8 23 23 Bd 1 1
C 63 ♀
I 9:63 14
III 24 9:62
III 8 53 52 Bd. 2
K 69 ♂
I 2 6:59
II 6 52 53 Bd. 2
III 7 5:59
C. 72 ♀
I Bd 1 30 5 5 13 5 5 8
II 4:62 4
K. 88 ♀
I 7 56 54 Bd 1
I 4:64 11
C 32 ♀
II 8 54 54 Bd 1
C 41 ♀
II 5 55 56 Bd 1

It should be noted by way of summary that in all cases in which the mother has a band anomaly, one or more offspring of a set have usually a similar band anomaly; but sometimes a scute anomaly is found to be the expression in the offspring of a maternal band anomaly. In several cases where there are band anomalies in the offspring a scute anomaly is found in the mother.

It has been shown clearly in several sets of offspring that there is an extremely close genetic relation between band anomalies and anomalies of single scutes. The two types of anomaly are merely more or less extensive expressions of the same genetic factor. Whenever a mother shows either type of anomaly, one or more offspring show one or the other type. The doubling factor is therefore strikingly dominant in the Mendelian sense. When we find sets of fetuses that show anomalies and the mothers of these show no anomalies, we must conclude that the anomaly is paternal, for a dominant factor in the mother, would appear phenotypically if present genotypically. It is interesting to note that almost the same number of sets have normal as have anomalous mothers.

Anomalous Offspring of Normal Mothers.

There are in the present collection 14 sets of fetuses showing band anomalies, the mothers of which showed neither band nor scute anomalies. In some ways these sets are better for our purposes than are those derived from anomalous mothers, because we can be sure of the uniparental character of the inheritance. Since the mothers show no anomalies, those in the offspring, being unquestionably inherited, must have come from the father. It would be highly interesting to know the conditions in the fathers, but the latter are inaccessible. Since there is no sex difference with regard to the anomalies, we may assume, however, that the same state of affairs would be revealed for the paternal as that made out for the maternal relation.

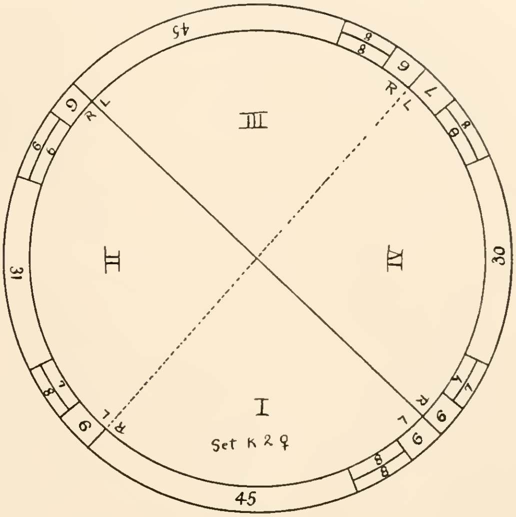
Some of the most interesting cases illustrating the symmetrical distribution of anomalies, intra- and inter-individually, are found among the offspring from normal mothers, and I shall give a complete tabulation of these sets, calling attention in the text only to certain of the more striking conditions, or to situations differing from those dealt with in the sets derived from anomalous mothers.

Set K. 2, ♀ *(Fig. 13).*

This set perhaps better than any other shows the phenomenon of symmetry reversal, or mirror-imaging among the quadruplets.

Fetuses I. and III., which face one another across the vesicle, and are the secondary bud derivatives of II. and IV. respectively, are exact symmetrical reversals of each other, I. having the anomaly only on the left and III. only on the right.

Fetuses II. and IV., the two primary bud individuals, both show the anomaly bilaterally, but are also reversals, in that II. has the more extensive doubling 9–9 on the right and IV. has the more extensive doubling 9–8 on the left. It is interesting to note that the primary individuals II. and IV. are bilateral in their expression of the anomaly, and that the secondary individuals I. and III. are unilateral.



13

Set A. 64, ♂ (Fig. 14).

This set is remarkable for the striking identity of detail exhibited by the anomalies of the different fetuses.

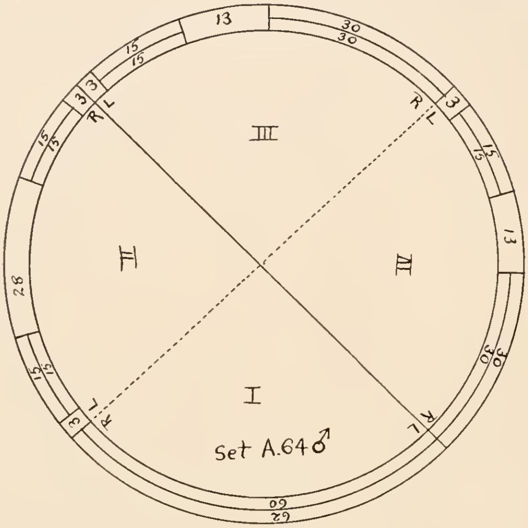
Fetus I. has both halves of band 1 completely double.

Fetus II. has both halves partially double and exactly bilaterally symmetrical, the doubling starting 3 scutes from the margin on each side and extending for 15–15 scutes.

Fetuses III. and IV are both exactly like II. on the left side and exactly like I. on the right.

There is exactly the same amount of doubling in I. plus II.

as there is in III. plus IV., but the distribution of the fully doubled and incompletely doubled half bands is different. In the case of the left-hand pair I. and II., I. gets both completely doubled halves and II. gets both incompletely doubled halves; while in the case of III. and IV. both get a completely and an incompletely doubled half. There is no symmetry reversal in this set.



14

Set A. 96, ♂ (Fig. 15).

Fetus I. has band 1 double except for 5 single scutes on the right margin.

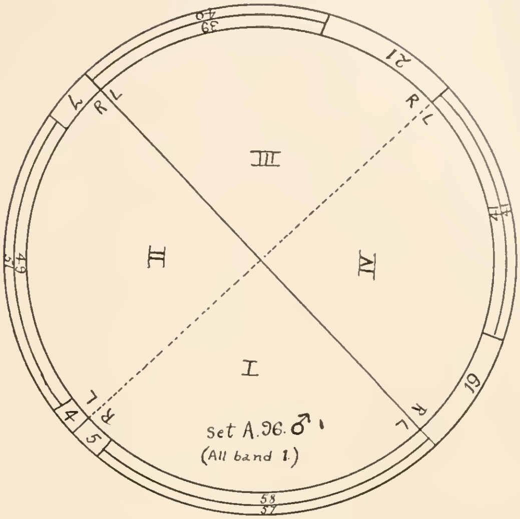
Fetus II. has a similar anomaly bilaterally.

Fetuses III. and IV. are very much alike but differ from I. and II. in the extent of the single part of the band, which is much longer than in the other pair. The pairing of fetuses is very clear, but no symmetry reversal is shown. One of four fetuses (II.) is bilateral.

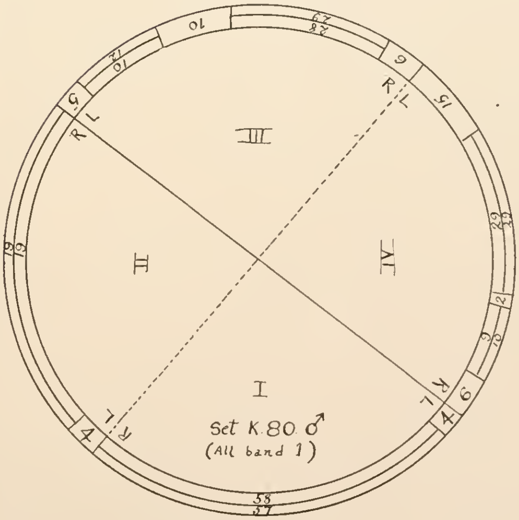
Set K. 80, ♂ (Fig. 16).

Fetuses I. and II. are practically identical. In both band 1 is doubled, except for four single scutes on the left.

Fetuses III. and IV. show the incomplete doubling bilaterally


40
39
21
7
R L
R L
III
II
IV
41
41
49
37
I
19
L R
R L
4
5
Set A.96. ♂
(All band 1.)
58
57

15


R L
R L
III
II
IV
I
R L
L R
Set K.80. ♂
(All band 1)

16

with a larger and smaller series of double scutes arranged on opposite sides. Fetus III. has the long series 28–29 six scutes from the right, while fetus IV. has a series of 29–29 scutes 15 scutes from the left and extending past the middle. This is an imperfect reversal of symmetry. There is a better reversal in connection with the short series of double scutes, for in III. the series of 10–12 scutes is situated 5 scutes from the left, and in IV. the series of 9–10 scutes is situated 6 scutes from the right.

Set A. 101 (Table B2) has the anomaly confined to one pair of fetuses (I. and II.) and the expression is very different in the two. It is well to note that although the doubling is nearly complete in II. it starts after 6 single scutes at the margin, as is the case on both sides of I., which shows a remarkably exact bilateral symmetry.

In the remaining sets, K. 8, K. 12, C. 90, C. 63, C. 69, C. 72, C. 88, C. 32 and C. 41 (Table B2 and 3), band doubling is found in only one fetus of a set. In most sets however scute anomalies, which are usually rather definitely related to the band anomaly, are present in one or more fetuses. Set K. 8 is remarkable for the large amount of reduplication down the axis of scute anomalies, for example fetus III. has double scutes in bands 1, 3, 5, 7, and 8, those on bands 5 and 7 being reversals of each other. It is clear that all of the double scutes are in the same region of the band as is the double series in fetus I., and the majority are on the same side of the body.

The remaining sets show nothing that has not already been seen in sets previously described.

Summary and Conclusions.

Band anomalies are of the nature of irregularities in the normal regular rows of scutes that make up the typical band, and consist of more or less extensive regional doubling of rows of scutes in a band. This condition is typical for the non-banded parts of the armor but quite rare in the banded regions. It is practically confined to the band or bands nearest the non-banded region. Band anomalies occur in only about three per cent. of individuals and an examination of over two thousand adult individuals taken at random shows no duplicate anomalies.

These anomalies are strongly inherited but are subject to more or less modification. Sometimes a band anomaly unilaterally placed may be inherited unilaterally but with a reversed symmetry to that of the mother, or it may be bilateral. Sometimes an extensive doubling is inherited both as a similar band doubling, and as a single double scute in the individuals of a single polyembryonic set of offspring. Contrariwise a double scute in the mother may be inherited as a more or less extensive unilateral or bilateral band doubling. The peculiarity may also be reduplicated down the primary axis in two or more bands.

Frequently in unilateral anomalies the different fetuses of a set show reversed symmetry or mirror-imaging, but it is even more common to find the unilateral anomaly on the same side of most of the individuals, or bilaterally in one or more of them.

In a number of cases the anomalies in different fetuses of a set are so strikingly identical as to indicate a rigid predetermination of the details of the character, but in other cases there appears to be only a predetermination of a generalized anomaly that expresses itself to a greater or less extent in the various embryos. In terms of Mendelian inheritance we may say that an anomaly factor is inherited as a dominant character, but its distribution among the fetuses of a set and its location and extent are due to varying ontogenetic or epigenetic factors.

The distribution of these inherited anomalies among the various fetuses of the sets furnishes much interesting data, which, together with data on the inheritance of double scutes, to be presented subsequently, furnishes the basis of a discussion of several general questions and especially those involved in the concept of organic symmetry.

This subject is reserved for a subsequent paper of the present series.

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FACTORS CONCERNED IN THE PRODUCTION OF MITOSIS IN ORGANISMS DISPLAYING CELL CONSTANCY.[1]

H. J. VAN CLEAVE.

The condition of absolute identity in cellular structure found in individuals of many species of Metazoa has led the writer to undertake an analysis of the factors governing mitosis in these forms. Loeb ('12: 4) has called attention to the fact that the first attempt to reduce the phenomena characteristic of life to purely physico-chemical terms is found in the works of Lavoisier and Laplace ('80) which indicated that the heat produced in the body of a warm-blooded animal equalled that given off by a burning candle when the amounts of carbon dioxide produced in the two instances were equal. These results have been so much amplified by later workers that today no one doubts that the general processes having to do with the phenomena of metabolism are identical with the so-called purely physical and chemical reactions occurring outside the living body. In fact while metabolism, movement, and irritability are generally granted as probably due to the workings of the same principles that govern the inanimate realm the fourth of the vital properties, that of reproduction, is in great part still unexplained. No problem has offered more secure harbor for a final possibility of the presence of some supernatural force than has this one dealing with the processes of reproduction. The statement of Davenport ('08: 1); "The vital processes are chemical processes, taking place in a highly complex, very unstable, constantly changing substance, whose activities we call life," embodies the modern conception of the nature of life phenomena in general, but no attempt has been made to show the actual possibility of explaining all life processes on this basis.

An analysis of the possible factors determining or directing the course of nuclear and cell division is extremely difficult on account of problems involved in eliminating factors until but a single causal agent is operative. However, of the possible factors involved in the production of mitosis and of cleavage two groups are clearly recognizable, namely: (1) Environmental factors, and (2) Internal factors. Any change which occurs within the living organism must find explanation upon the basis of one of these two groups of factors or upon the basis of a combination of them. It is the purpose of this paper to show the relationship of these two groups of factors in the production of mitosis among those organisms which are made up of a fixed number of cells.

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[1] Contributions from the Zoölogical Laboratory of the University of Illinois, No. 43.

1. Environmental Factors.

If environmental factors either caused or directly controlled the phenomena of mitosis the fact of cell constancy could not exist, for no two individuals, and more strongly no two groups of individuals even of the same species, develop under absolutely the same environmental conditions. Consequently if environmental factors were the limiting factors in mitosis no two individuals would of necessity contain the same number of cells. It is generally granted that the mitotic process may be accelerated or retarded through the application of purely external stimuli. At least in those organisms made up of a fixed number of somatic cells such an acceleration or retardation of the mitotic process could result in nothing beyond a modification of the normal *rate of the process* and could in no wise be considered as a direct factor in the determination of the *extent of the series* of mitotic divisions during the cleavage stages and in subsequent development. If the role of temperature, for instance, be examined as a possible controlling factor in the mitotic process it becomes apparent that if this alone and directly controlled the number of mitotic divisions through which the developing organism should pass there could be but slight possibility of any two individuals having identical numbers of somatic cells. For under conditions of nature no two individuals are at all times during their development under absolutely identical temperature relations. A similar variability of conditions for the development of different individuals exists in the case of almost all of the other environmental factors which might be considered as directly influencing the process of mitosis.

Furthermore if environmental conditions could directly control and determine the mitotic process, cell division would continue indefinitely through the life of the organism as long as the external conditions remained favorable for it. Again the data brought from the field of cell-constancy show the impossibility of such factors being operative in a controlling manner. In the development of any organism or part of organism the powers of reproduction of most of the somatic cells are restricted to definite periods in the early stages of development, usually preceding the introduction of histological differentiation. Thus in the genus *Eorhynchus* the writer ('14: 280) has shown that in no instance did he find any adult worm displaying abnormal numbers of somatic nuclei or presenting any evidence whatever of further division of the somatic nuclei after the adult body form had been attained. These observations were upon over two hundred individuals collected during a period of four years at various localities and at various seasons of the year.

In conclusion, if the determination of the mitotic process were to find its explanation in terms of environmental factors, or to a continuous production of combinations of environmental factors during the process of development, then in every instance the number of mitotic divisions a fertilized egg would undergo would be a direct resultant of the complex of environmental factors operative during its process of development. At least in those forms with a high degree of cell constancy it seems obvious that purely environmental factors have but one relationship to the process of mitosis and that consists in the modifiability of the rate, either as an acceleration or as a retardation. In this respect purely environmental factors have the same general effect upon the process of mitosis as they exert upon purely physical and chemical reactions.

2. INTERNAL FACTORS.

Our incomplete knowledge of the finer structure of the cell, and of the nucleus in particular, make it impossible to associate the control of the mitotic process directly with any structure or chemical bodies found in the cell. However on the basis of facts pointed out earlier in this paper it seems certain that since there is no possibility of environmental factors acting as the controlling element in mitosis the ultimate cause of the process must be sought within the cell. In this connection invaluable support is found in the field of experimental embryology. Morgan ('95, '01, and '03) and Driesch ('98 and '00) have both shown that in the embryos of echinoderms developed from isolated blastomeres of the two cell stage the number of cells present at any point in the development is approximately half of the number present in a normal embryo. Similarly from one of the blastomeres of the eight cell stage the gastrula is composed of only one eighth the number of cells found in the normal gastrula. Loeb ('06: 59) interprets these results as supporting the hypothesis of Sachs ('93 and '95) which regards the factors determining cleavage controlled by the ability of each nucleus to gather around itself and control a definite amount of protoplasm. Yet what determines the amount of protoplasm present in the developing individual? The cytoplasm is constantly being replaced through the processes of anabolism which experiments with enucleated cells have shown to be under the control of the nucleus. Consequently it seems that the view just stated comes not much short of being an argument in a circle. Does the amount of cytoplasm determine the number of nuclei that are to be formed or is the numerical relation of nuclei to cytoplasm a mutual one brought about not through the influence of either cytoplasm or of nucleus but through some fundamental factor which determines the number of nuclei and at the same time indirectly the amount of cytoplasm that is to be formed? The writer interprets the data of Morgan and of Driesch in an entirely different manner. If by the first division of the egg there are set apart two units, each of which has the possibility of developing into a given number of cells by the process of mitosis and this tendency is retained, even though the two units become separated, it seems logical to conclude that within the fertilized egg there are resident potencies which through the process of mitosis become divided between the two daughter nuclei of the first and then of each succeeding generation of cells.

As to the nature of this partition with each mitotic division two explanations present themselves. According to the simplest of these mitosis may be the result of the direct chemical activity of certain substances, present in the fertilized egg, which become used up in the mitotic process so that each cell of the two cell stage receives an equal amount of the substance present in the fertilized egg after the amount necessary for the first mitotic division has been eliminated. On this hypothesis with each succeeding mitotic division the materials resident in the developing embryo become partitioned and dissipated in the process of development. Thus if x equals the entire amount of the material for the execution of the mitotic process present in the fertilized egg, and a the amount required for the realization of the first mitotic division then each cell of the first cleavage would receive $(x - a)/2$. In this manner each succeeding division would reduce the amount of the material present until the amount apportioned to each cell would be less than the amount required for the execution of the mitotic process, thus bringing about an automatic check upon the course of the series of nuclear divisions. The following objection to such a hypothesis shows its weak point. Upon this basis each blastomere of the early stages would be required to produce the same number of cells, a supposition which the facts of cell lineage do not support.

The second explanation of the method of control over the number of divisions of the nucleus seems more natural and does not convey so much of the idea of predestination, in that it requires less emphasis upon the inherent qualities of the fertilized egg. According to this hypothesis mitosis is just as markedly a result of chemical processes going on within the egg as indicated above but the individual cells may in varying degrees retain the power of synthesizing the materials necessary to initiate and carry out the mitotic division of the nucleus. In the application of this explanation the check to the course of the nuclear divisions may come as the result of an accumulation of materials within the cell, probably as metabolic by products, which serve to retard and finally to prohibit the chemical activity incident to nuclear division. Thus at the end of any definite period of physiological activity the organism will be composed of a definite number of cells and so upon attaining maturity will have a fixed number of somatic cells which are unable to divide farther on account of the presence of the inhibiting materials. It must be remembered that throughout this discussion principles are being laid down for the determination of the mitotic process in organisms or parts of organisms displaying cell constancy. The objection involving the variability in the number of cells arising from the early blastomeres, cited in connection with the discussion of the influence of environmental factors, finds no grounds here. In fact it lends its support to this second hypothesis. If the ultimate check to the mitotic process comes as the result of an accumulation of metabolic by products, then the *rate* of the nuclear division, which unquestionably may be influenced by environmental factors, would tend but to be directly proportional to the rate of the accumulation of the inhibiting elements, though the actual amount of the inhibitors necessary to terminate the series of mitotic divisions would remain the same. Evidently the accumulation of the inhibitors proceeds unequally in various tissues of the metazoan body. The degree of the differentiation of the cell and, in all probability connected with this, the nature of the cell membranes, determines the rate of the accumulation of the inhibiting factors.

The impossibility of explaining the phenomena of cell constancy on the basis of mitotic control by environmental factors and the facts from experimental embryology in their bearing upon the control of mitotic division by internal factors, lead to the evident conclusion that during the development of cell constant forms the mitotic process is controlled and determined from within the cell but its rate may be regulated by factors which are considered purely environmental.

Conclusions.

1. At least in those forms displaying a high degree of uniformity in the number and arrangement of their component cells and nuclei, environmental factors such as ordinarily influence physical and chemical changes play no direct part in the determination of the number of mitotic divisions of the somatic nuclei.

2. The only influence of normal environmental factors in these

forms consists in the modification of the rate of the process of mitosis.

3. The direct control of the mitotic process must be sought in the chemical activity going on within the cells of the developing individual.

4. Experimental evidence indicates that with each cleavage of an egg with determinate cleavage there is retained a definite relationship between the number of any given cleavage and the total number of cleavages that the embryo would undergo even in those cases where the blastomeres become isolated in the early stages of development.

5. The foregoing would indicate clearly that within the cells derived from the fertilized egg there are present factors or potencies which exert direct control over the number of mitotic divisions which shall ensue.

6. The fact that not all of the blastomeres of the early cleavages produce the same number of cells indicates that the number of cells produced must be controlled by conditions developing as the process of development progresses rather than by the partition and distribution of some definite materials present in the egg at a time prior to the first cleavage.

7. In tissues which retain the power of continued mitotic division, as for example in the formation of the germ cells and in tissues which have widely varying numbers of cells, the explanation of the inconstant nature of the numbers of cells produced may be sought in the acquisition of the power of eliminating from the cell those materials which in the course of the process of metabolism tend to accumulate and serve as inhibitors to the mitotic process.

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THE PRODUCTION OF MALES AND FEMALES CONTROLLED BY FOOD CONDITIONS IN THE ENGLISH HYDATINA SENTA.

DAVID D. WHITNEY.

It has been found recently in an American parthenogenetic strain of the rotifer, *Hydatina senta*, from New Jersey that a continuous diet of the colorless protozoan flagellate, *Polytoma*, causes practically all females to be produced.[1] This production of only females can be maintained in this manner through generation after generation for several years. If, however, the diet is suddenly changed to a green protozoan flagellate, *Chlamydomonas*, that is in an active state, males can be produced in great numbers.

It has been suggested that perhaps this phenomenon of the regulation of the two sexes by food conditions is peculiar to this particular strain of New Jersey *Hydatina senta* and is not an universal characteristic of the species. Fortunately it has been possible to test this hypothesis on an English strain of *Hydatina senta* and some very clear and conclusive results have been obtained.

I am greatly indebted to A. F. Shull for the stock of English rotifers with the accompanying data. "The English line was received from Mr. C. F. Rousselet, who collected them as resting eggs in mud at the bottom of a duck pond in England in August, 1912. They were sent to me about November 1, 1912, in dry dirt. The first ones hatched a few days later and from them the line sent to you has been reared by parthenogenesis ever since. As they were reared there was a generation about every three days and so you have about the 270th generation." The stock of this English line was received from Dr. Shull on January 7, 1915.

The females of this strain produce fewer offspring in the same period of time than the females of the New Jersey strain. This is probably due to the fact that the former strain had been reproducing parthenogenetically for several years and had become weakened whereas the latter strain was developed from a resting egg in November, 1914, and at the present time is as vigorous as at first.

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[1] *Jour. Exper. Zool.*, Vol. 17, November, 1914.

The method of making the media and rearing the two kinds of protozoan food cultures has been given in detail in a former paper and will not be repeated here, excepting a few additional words about the green food. When the *Chlamydomonas* had been 2–3 weeks in the same media the individuals were large and more or less quiescent. In this condition they were only moderately effective although in one or two instances some that had been two to three months in the same media were very effective. When they had been in new bouillon media for 2–7 days with considerable sunshine at a temperature of about 28° C. they were of various sizes and seemed to be the most effective. However, there seems to be a considerable amount of chance in getting the *Chlamydomonas* in the optimum condition for every experiment. This probably explains the varying percentages of the male-producing females obtained in the different experiments.

As it seems that the effect of a uniform diet on this rotifer has not been sufficiently emphasized the following Table (I.) concerning this point in three pedigreed races has been compiled from the records in an earlier paper.[1] This shows that a constant and uniform diet of *Polytoma* has repressed the production of males for nearly 300 generations. The long period of time

Table I.

Showing that a Constant and Uniform Diet of the Colorless Protozoa, *Polytoma*, Represses the Production of Male-producing Females and Causes the Production of Female-Producing Females.

Kept on a Uniform Polytoma Diet.

Race.	Generation.	♂♀	♀♀	% ♀♀	Time.
A........	1–288	92	2,565	96+	22 months
B........	1–181	8	1,731	99+	14 months
C........	1–288	0	2,749	100	22 months

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[1] Biol. Bull., Vol. 22, March, 1912.

and the large number of generations through which these races were reared and observed would seem to warrant the reliability of the results.

Table II. contains the results of some new observations made in November and December of 1914. The main point of interest in it is that it shows at what place or stage after the diet has been changed females may be isolated that will produce a high percentage of male-producing daughters. Adult females (mothers) that were put into the new diet of green food produced in it, during 1–12 hours, 15 per cent.–60 per cent. of male-producing daughters, but the same adult females in the second 12 hours produced a much higher proportion of male-producing daughters, usually from 80 per cent.–100 per cent. In most of the experiments the adult females were taken out of the green food after they had been in it 12 hours, in more or less of sunlight, and placed in filtered culture water from a general stock battery jar

TABLE II.

AS A CONTROL FOR TABLE III. AND ALSO SHOWING THE HIGH PERCENTAGE OF MALE-PRODUCING DAUGHTERS PRODUCED AFTER THEIR MOTHERS HAD BEEN TRANSFERRED FROM THE *Polytoma* DIET TO THE GREEN *Chlamydomonas* DIET FOR 12 HOURS.

Experiments During November and December 1914 on a New Jersey Strain of Hydatina senta.

	Control Reared and Continued on a *Polytoma* Diet.				Adult ♀♀s from the Control Reared on a *Polytoma* Diet Transferred to a *Chlamydomonas* Diet.						
Experiment.	♀♀ Mothers.	Daughters.			♀♀ Mothers.	Daughters Produced During First 12 Hours.			Daughters Produced During Hours 12–24.		
		♀♀	♂♀	%♂♀		♀♀	♂♀	%♂♀	♀♀	♂♀	%♂♀
1	10	10	0	0	6	14	20	59+	0	22	100
2	10	10	0	0	5	33	6	15+	14	12	46+
3	10	10	0	0	5	24	6	20	8	32	80
4	10	10	0	0	5	No record			7	17	70
5	10	10	0	0	10	"	"		31	61	66*
6	10	10	0	0	5	7	13	61+			
7	10	10	0	0	40	No record			9	104	92+
8	10	10	0	0	40	"	"		24	126	84
9	10	10	0	0	40	"	"		30	129	81+
10	10	10	0	0	10	"	"		1	39	97+
	Control continued through 30 additional generations										
	300	300	0	0							
Total....	400	400	0	0	166	78	45	36+	124	542	81+

in which various protozoa and rotifers were living. They were left in this filtered water for about 12 hours, during the night, without food. During this time each female laid 3–4 eggs and the next morning the old females were taken out, *Polytoma* food was added, and the eggs allowed to hatch. Several hours later the young females that hatched from these eggs were isolated in separate watch glasses and fed *Polytoma* and a species of a small *Euglena*.

In Table III. this same fact is shown again to be true for the English species. However, this is a relatively minor point and may only be of importance to any one wishing to obtain cytological material.

Table III.

Showing that When the English Strain Was Subjected to a Uniform and Constant Diet of the Colorless Protozoa, *Polytoma*, Only Female-producing Females Were Produced but that When it was Subjected to a Sudden Change of Diet from the *Polytoma* to a Green Protozoa, *Chlamydomonas*, as High as 85 Per cent. of Male-producing Females Was Produced.

Experiments During January and February 1915 on an English Strain of Hydatina senta.

Experiment.	Control Reared and Continued on a *Polytoma* Diet.				Adult ♀♀s from the Control Reared on a *Polytoma* Diet Transferred to a *Chlamydomonas* Diet.						
	♀♀ Mothers.	Daughters.			♀♀ Mothers.	Daughters Produced During First 12 Hours.			Daughters Produced During Hours 12–24.		
		♀♀	♂♀	% ♂♀		♀♀	♂♀	% ♂♀	♀♀	♂♀	% ♂♀
1	10	10	0	0	5	7	4	36+	4	14	77+
2	10	10	0	0	5	8	2	20	5	5	50
3	10	10	0	0	5	4	3	42+	9	3	25
4	10	10	0	0	5	14	4	22+	8	4	33+
5	10	10	0	0	20	29	11	27+	16	27	62+
6	10	10	0	0	20	38	8	17+	7	40	85+
7	10	10	0	0	15	No record			15	13	46+
8	10	10	0	0	20	30	18	37+	No record		
9	10	10	0	0	90	No record			50	76	60+
	Control continued through 20 additional generations										
	200	200	0	0							
Total....	290	290	0	0	185	130	50	27+	114	182	61+

If Table III. is compared further with Table II. it is evident that they are very similar in all points with the exception that the percentages of male-producing females in Table III. are somewhat lower than those in Table II. However, both tables show that a continuous and uniform diet of *Polytoma* causes only female-producing daughters to be produced while a sudden change of the food from *Polytoma* to *Chlamydomonas* causes male-producing daughters to be produced in great numbers.

SUMMARY.

1. A uniform diet of the colorless protozoan flagellate, *Polytoma*, continued for 22 months through 288 generations practically suppressed the production of males and caused only females to be produced in the rotifer, *Hydatina senta*.

2. After the adult females had been transferred from the diet of *Polytoma* to the diet of *Chlamydomonas* for 12 hours they produced a higher percentage of male-producing daughters than they did during these 12 hours.

3. The sudden change of food from a constant diet of *Polytoma* to a diet of the green *Chlamydomonas* caused male-producing daughters to be produced in the English *Hydatina senta* about as readily as in the New Jersey *Hydatina senta*.

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MICROTÆNIELLA CLYMENELLÆ, A NEW GENUS AND NEW SPECIES OF COLONIAL GREGARINES.

GARY N. CALKINS.

While examining the gut contents of marine annelids at Woods Hole during the month of June, 1914, a number of new gregarines were discovered. The majority of these were ordinary types which could not be placed systematically without knowledge of the sporulation stages. One form, however, found in the digestive tract of *Clymenella torquata*, deserves mention because of its remarkable novelty.

To obtain the material, the worms were opened along the mid-dorsal line; sections of the digestive tract about half an inch long, were removed and teased in salt solution on cover glasses. The material thus prepared was examined while fresh, and, if interesting, was then fixed in sublimate acetic and stained. In this way I have obtained more than a hundred specimens of the curious organism described here.

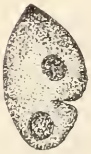
The name *Microtæniella* is given because of the Tænia-like structure of the organism (Figs. 1–5). The initial single cell resembles a scolex and the chain of cells, formed partly from the initial cell and partly by cell division of the daughter individuals, resembles a chain of proglottids. In sporozoan terminology these may be termed the primite and satellites respectively, although this involves some elasticity in the use of the terms primite and satellite which are usually employed to designate the primary and secondary individuals in a chain of gregarines formed by secondary association.

In the living state, the organism is colorless but with the characteristic dense protoplasmic structure of the gregarines. The average dimensions of the chain are 84 μ in length and 15 μ in width, the size of mature chains varying but little from the average.

The earliest stage found was an initial cell with two nuclei and the beginnings of a septum cutting off a first satellite (Fig. 1).

All intermediate stages between this young form and stages pictured in Figs. 4 and 5 were found. Each satellite contains a single nucleus except in the division stages and in the terminal cells of the oldest satellites.

New cells are continuously given off by the primite; these reproduce by division, the plane of division being at right angles to the long axis of the aggregate for the first one or two divisions. In some chains the successive individuals furnish almost every stage for an ideal diagram of cell division (Fig. 3). In the older satellites the plane of division changes from transverse to longitudinal in respect to the long axis of the chain. In this way two terminal chains may originate, but such branch chains never become very long owing to further development and to separation of the satellites from the parent colony.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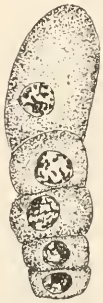
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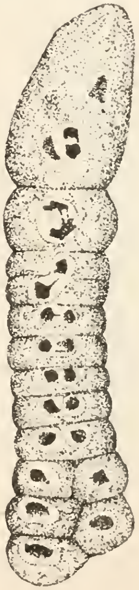
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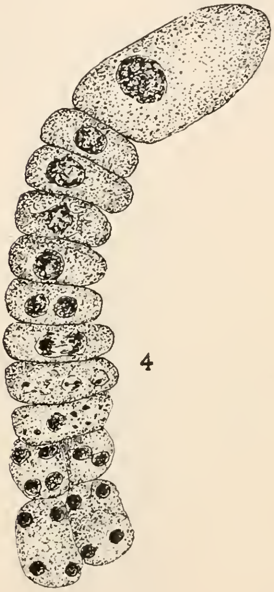
After from eight to ten satellites have been produced thus by division, the terminal cells become more spherical, their nuclei divide twice, and without cytoplasmic division, giving rise to a terminal group of cells each with four nuclei (Fig. 4). This stage must persist for some time for several specimens were found without evidence of further advance. In one specimen (Fig. 5), a group of terminal satellites, evidently the result of this phase of activity in two branched terminal chains, were present. Ultimately, division planes appear in the cytoplasm around each of the four nuclei, and four naked cells are produced. The fate of these cells is not known; possibly they become new primites and repeat the cycle, but more probably, they are gametes which unite to form sporoblasts characteristic of the gregarines. I shall try to work out their further history during the coming June.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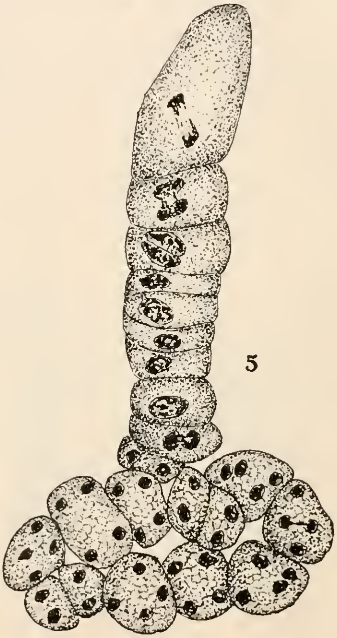
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This rather paradoxical organism has given me a great deal of trouble. Is it a metazoön or a protozoön? Is the "individual" the entire chain or is it a chain of individuals? Although the aggregate has a definite morph and a definite ontogeny I am inclined to regard it as a chain of individuals in which original reproduction by division has become modified to asymmetrical division or budding in the primite, but is retained by the satellites. Originally, it may be assumed, the daughter individuals became separated and after a few divisions finally gave rise to sporonts. If the entire chain is regarded as the individual we are met by the same difficulty which confronts us in connection with the colonial flagellates.

The possibility of this being a plant form has not been overlooked. The absence of definite walls together with the method of cell division and terminal cell changes are opposed to what is known of types in the group of fungi. To make sure, however, I showed the specimens to my colleague Professor R. A. Harper who gives me permission to quote him to the effect that he finds nothing in the structure or the history of this organism that would justify him in placing it with plant forms.

Amongst protozoa, nothing, so far as I am aware, has been described of like nature. Leger's *Tæniocystis* is a gregarine with numerous septa and with external annulations which give it the appearance of a *Tænia*, but it is a single cell and has a single nucleus. The absence of motile organs in the present form, and its mode of reproduction, leave no grounds for placing it otherwise than with the sporozoa, and here the only possible place for it is with the gregarines. There is some evidence that the often sharply pointed end of the primite is the attaching portion, but no stage showing such attachment has been found in the smears.

I would classify *Microtæniella* as a colonial parasite belonging to the order Gregarinida, suborder Schizogregarina, of which it should form a new subdivision.

Columbia University,
March, 1915.

THE BLINDNESS OF THE CAVE FAUNA AND THE ARTIFICIAL PRODUCTION OF BLIND FISH EMBRYOS BY HETEROGENEOUS HYBRIDIZATION AND BY LOW TEMPERATURES.[1]

(WITH 13 FIGURES.)

JACQUES LOEB.

I.

While many of the animals inhabiting caves are blind or have degenerated eyes, the same phenomenon is rarely found among animals that live in the open. At first sight this seems to suggest that the disuse of the eyes in the complete darkness of the cave has gradually led to the degeneration of the eyes and this idea seems at one time to have been widely accepted. In forming a judgment of the connection between the darkness of the caves and the blindness of cave dwellers we must remember that some of the cave dwellers have perfectly normal eyes. Thus Eigenmann, to whom we owe the most thorough study of this subject, points out that of the four species of salamanders living habitually in North American caves two have apparently quite normal eyes. They are *Spelerpes maculicauda* and *Spelerpes stejnegeri*. Two others living in caves have quite degenerate eyes, *Typhlotriton spelæus* and *Typhlomolge rathbuni*.[2] If disuse is the direct cause of blindness we must inquire why *Spelerpes* is not blind.

Another difficulty arises from the fact that a blind fish, *Typhlogobius*, is found in the open (on the coast of southern California) in shallow water, where it lives under rocks in holes occupied by shrimps. One must again ask the question: How can it happen that in spite of the exposure to light *Typhlogobius* is blind?

The most important fact is perhaps the one found by Eigenmann in the fishes of the family of Amblyopsidæ. Six species of this group live permanently in caves and are not found in the open, while one lives permanently in the open and is never found in caves and one comes from subterranean springs. The one form which is only found in the open, *Chologaster cornutus*, has a simplified retina aside from having a comparatively small eye, in other words, its eye is not normal. This indicates the possibility that the other representatives which are only found in caves also might have abnormal eyes even if they never had lived in caves.

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[1] From the Rockefeller Institute for Medical Research, New York.

[2] Eigenmann, "Cave Vertebrates of America," Carnegie Institution Publications, Washington, 1909.

Through these facts the old idea becomes doubtful, namely, that the cave animals had originally been animals with normal eyes in which the disuse had led to a gradual hereditary degeneration of the eyes. Instead we must consider the possibility that in the blind cave animals as well as in the blind animals which live in the open the tendency towards blindness developed independently of presence or absence of light. From this point of view the tendency toward degeneration of the eye appears as a hereditary mutation comparable to the inherited glaucoma which is known in the human. Glaucoma is a form of blindness caused by the atrophy of the optic nerve in consequence of an increased intraocular pressure and this high pressure seems to be caused by a certain disturbance in the circulation in the eye. The fact that these patients are born with normal eyes and do not become blind until later in life shows that lack of light has no share in the development of this hereditary disease or mutation. The question then remains, how can we account under such an assumption for the fact that blind animals are so prevalent in caves and so rare in the open? We shall return to this question later on.

II.

We will now review briefly the literature dealing with observations and experiments on the influence of light on the formation of eyes. The fact that the eyes of mammals are formed in complete darkness (in the uterus) may serve at the outset as a warning against overestimating the effect of light on the formation of eyes. F. Payne[1] has raised sixty-nine generations of a fly

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[1] F. Payne, BIOL. BULL., XXI., p. 297, 1911.

(*Drosophila*) in complete darkness without noticing any changes in their eyes or in their sensitiveness to light.

Recently Uhlenhuth[1] has demonstrated in a very striking way that the development of the eye does not depend upon the influence of the light or upon its functioning. He transplanted the eyes of young salamanders into different parts of their bodies where they were no longer connected with the optic nerves. The eyes after transplantation underwent a degeneration which was followed by a complete regeneration. Uhlenhuth showed that this regeneration took place in complete darkness and that in salamanders, kept in the dark for 15 months, the transplanted eyes remained normal. Here the eyes were no longer in connection with the central nervous system, received no light, and could not possibly have functioned and yet they regenerated and kept normal. The degeneration which took place in the eyes immediately after they were transplanted was

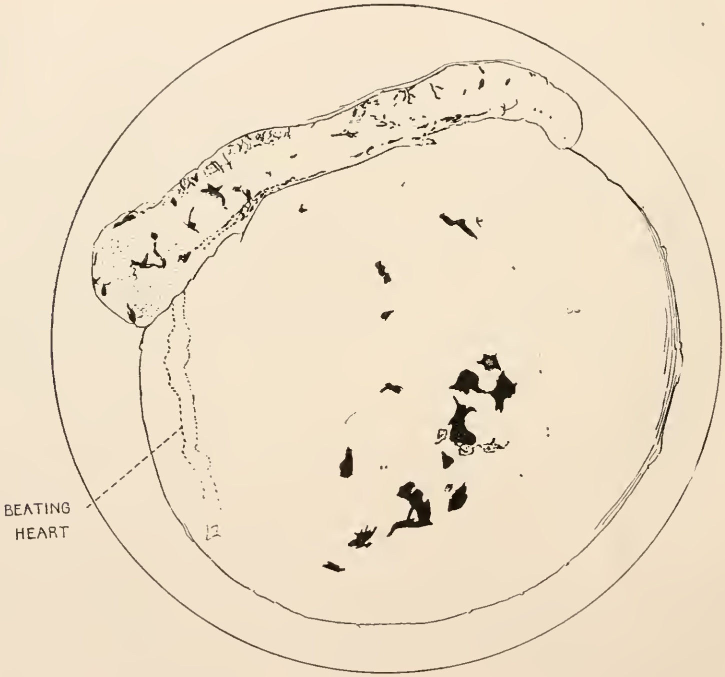

Fig. 1.

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[1] Read at the meeting of Anatomists at St. Louis, December 1914.

apparently due to the interruption of the circulation in the eye and the regeneration probably set in with the reëstablishment of the circulation in the transplanted organ.

The older literature had many observations which were assumed to prove an influence of light on development but the writer has shown in a former paper that these conclusions were not supported by the facts on which they rested.

The writer has for years paid special attention to the possibility that light might influence the formation of organs in animals, but he has succeeded in finding only one form in which such an influence can with certainty be demonstrated, namely the hydroid *Eudendrium.* In his experiments in Naples he had noticed that the polyps of this form regenerate better in the light than in the dark and in Woods Hole he could convince himself that the stems of *Eudendrium* cannot regenerate their polyps if kept permanently in the dark.[2]

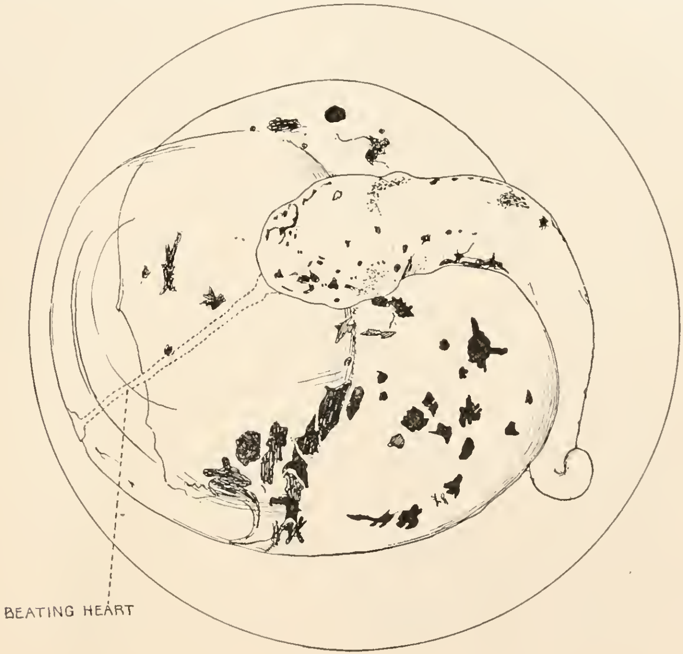

FIG. 2.

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[2] Loeb, "Über den Einfluss des Lichtes auf die Organbildung bei Tieren," *Arch. f. d. ges. Physiol.*, LXIII., p. 273, 1896; Goldfarb, *Journal Experimental Zoology*, 3, 129, 1906; 8, 133, 1910.

Kammerer[1] seems to be the only author who still takes it for granted that cave animals owe the degeneration of their eyes to lack of light, and his support for this view consists in the statement that five young cave salamanders (*Proteus*) developed larger eyes under certain (somewhat puzzling) conditions of illumination. In other cases the eyes of *Proteus* remained unaltered in the light. It would be advisable to make certain whether or not there are two varieties of *Proteus* and moreover it would be desirable to repeat these experiments on a larger scale.

The writer wishes to publish in this note the results of some experiments made in 1912, which prove that in the fish *Fundulus* it is comparatively easy to produce embryos with degenerated eyes by various means except by the one Kammerer holds responsible for this phenomenon, namely lack of light. The reader may in passing be reminded of the fact that in this form Stockard and later McClendon induced the formation of cyclo-

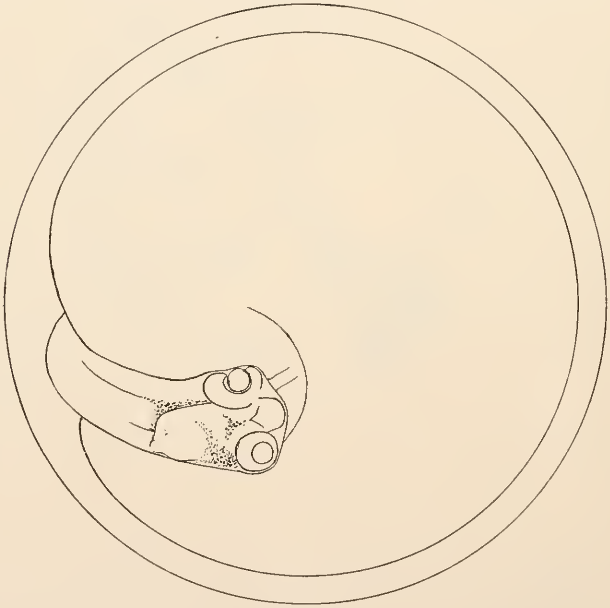

Fig. 3.

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[1] Kammerer, *Arch. f. Entwicklngsmechn. d. Organ.*, XXXIII., p. 350, 1912.

pean eyes by altering the constitution of the sea water (addition of magnesium salts or of alcohols and other narcotics.)[1] The writer's attention to an abnormal development of the eyes in this embryo was first attracted in his experiments on heterogeneous hybridization.[2] He noticed that among the anomalies noticeable in heterogeneous fish hybrids the lack of circulation was the most common, but that incomplete or abnormal development of the eyes was not infrequent. Such embryos often give the impression that they have no eyes at all, though in reality a histological analysis would probably show that some of the organs of the eye are present. But it is safe to say that the condition of the eyes is such as to make them unfit to form a retina image and for this reason we may call these embryos with abnormal (or apparently lacking) eyes blind; in the same sense in which this term is used in the case of the blind salamanders or

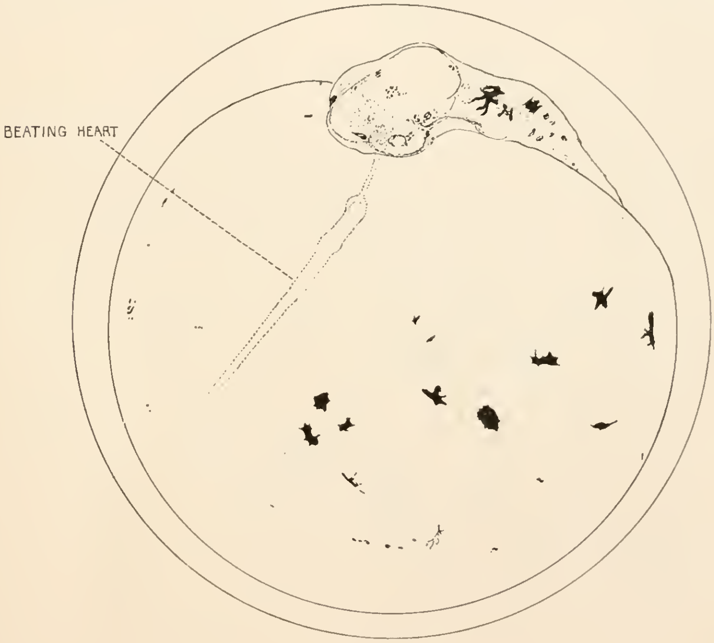

Fig. 4.

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[1] Stockard, *Am. Jour. of Anatomy*, 10, 369, 1910; *Jour. Exp. Zool.*, 4, 165, 1907; 6, 286, 1909; McClendon, *Am. Journ. Physiol.*, 29, 289, 1912.

[2] Loeb, "Heredity in Heterogeneous Hybrids," *Jour. Morphol.*, XXIII., p. 1, 1912.

fish found in caves which also have some of the elements of an eye but in a condition unfit for use. The term "blind fish" is therefore used in the following as synonym with imperfect or degenerated eyes.

III.

Blindness Produced through Heterogeneous Crossings.

Figs. 1 and 2 are camera drawings of hybrids between *Fundulus heteroclitus* ♀ and *Menidia* ♂. The first embryo is eight, the second fourteen days old. In neither of these two embryos are eyes noticeable, though of course a histological examination might have divulged rudimentary eyes. The embryos are in this respect to all appearances comparable to the blind cave fish or salamanders.

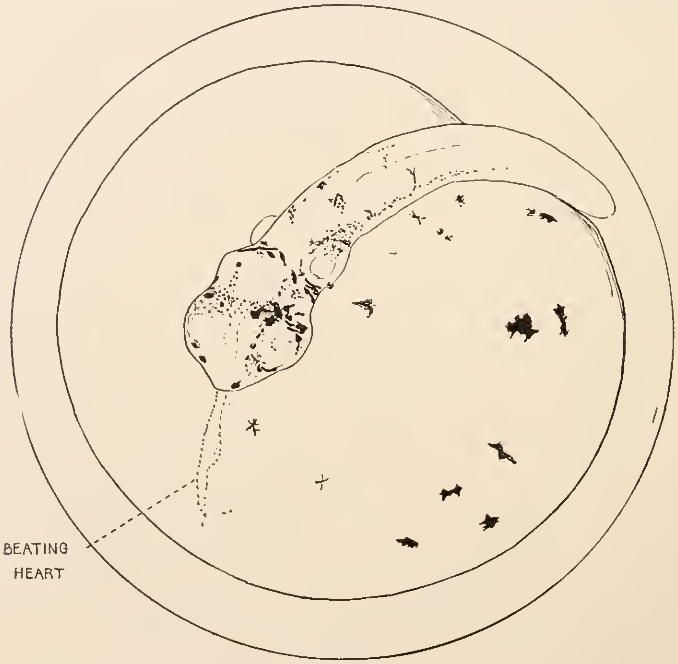

Fig. 5.

It is a known fact that certain blind animals have in an early stage a comparatively better developed eye than in later stages, *i. e.*, that the eye either stops developing early while the development of the rest of the body continues or that the eyes degenerate directly. The writer noticed not infrequently that the eyes of the cross between *Fundulus* and *Menidia* were normal in the beginning but appeared more abnormal the further the development progressed. We will illustrate this statement by two series of drawings. The first case is illustrated by Figs. 3 to 6. Fig. 3 shows the embryo four days old. The embryo has normally developed eyes with lenses. The embryo's heart, which is visible in front, was not yet beating and no pigment was formed. The same embryo is shown two days later in Fig. 4. The eyes instead of developing any further are less distinct. Fig. 5 is the same embryo two days later (eight days old). The eyes are hardly recognizable, it is difficult to decide without sectioning whether the lens still exists. The internal ear is distinguishable. The heart is pulsating.

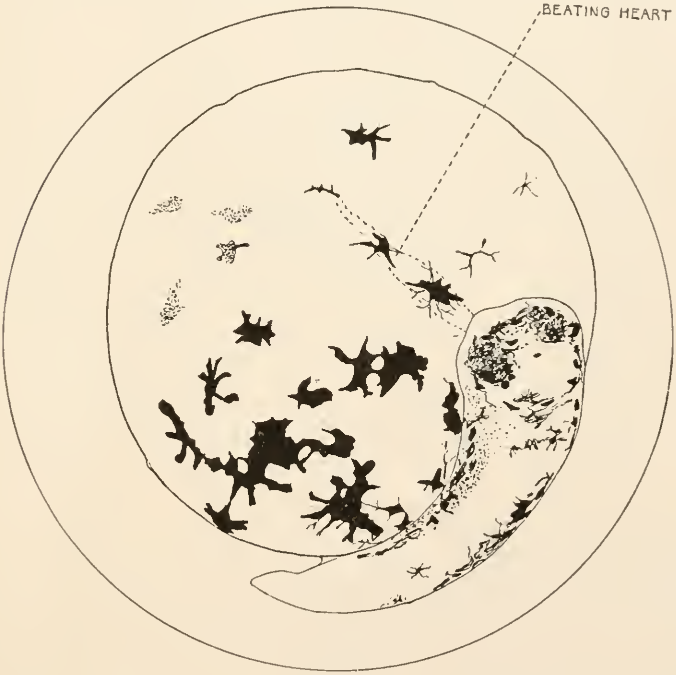

FIG. 6.

Fig. 6 shows the same embryo at the age of two weeks. Apparently the pigment is the only organ of the eye which is developed.

Figs. 7 and 8 illustrate the same fact, that a rather normal eye with a lens was formed while later on only a very imperfect eye remained. Fig. 7 shows the embryo when four days old with a lens and an apparently normal eye, while five days later (Fig. 8) no eye was perceptible.

IV.

BLIND FISH PRODUCED FROM PURE BREEDS OF FUNDULUS HETEROCLITUS.

The writer was under the impression that the abnormalities in the development of the heterogeneous hybrids in fish were due to the influence of the foreign sperm as in the similar case of the fertilization of the egg of the sea urchin with the sperm of the star fish, where the mortality is also enormous. In order to prove this assumption he tried to find a method which would allow him to produce embryos with abnormal eyes in the pure breeds of *heteroclitus*. He has already mentioned in a former publication that such abnormal embryos were obtained when

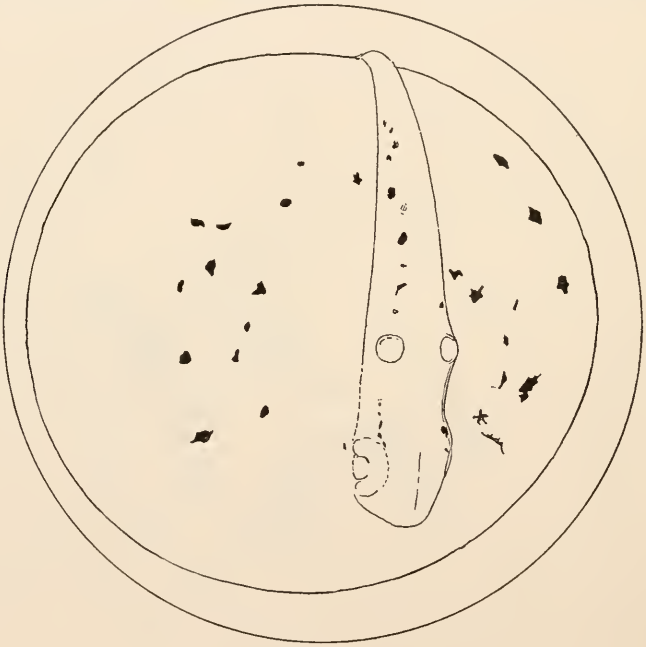

FIG. 7.

their development was retarded by putting them into sea water to which some KCN was added. Fig. 9 shows a blind embryo obtained in this way. The eggs were put immediately after fertilization into a weaker solution of KCN in sea water where they remained sixteen days. They developed slowly. The embryo was drawn two days after it had been put back into normal sea water. The heart-beat and circulation were established. This method of producing blind embryos is not reliable and need not be discussed any further.

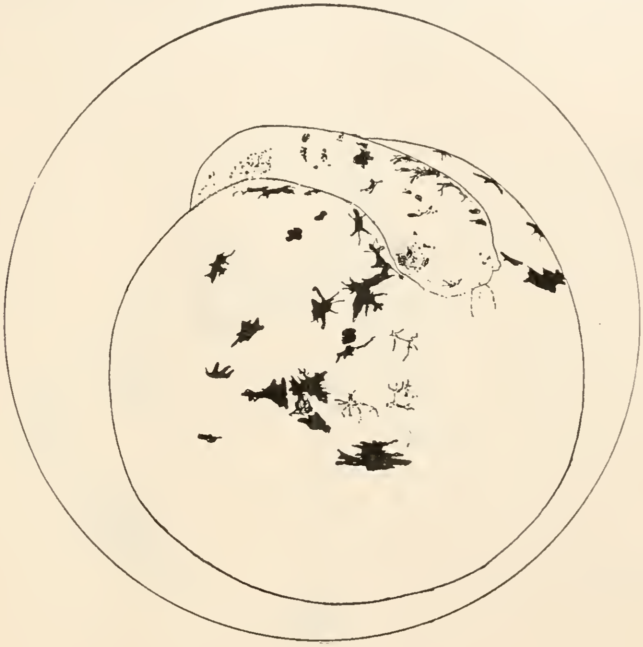

Fig. 8.

A better method was found by exposing the newly fertilized eggs to a low temperature (from between 0 to 2° C.) for some time. The writer found that the egg of *Fundulus* can be put for weeks into such a low temperature after the embryo is once formed, without any injury to the latter. As soon as it is put back to room temperature it continues to develop. This corresponds with the idea that the low temperature only retards the chemical reactions underlying development. If, however, the egg of the same fish is exposed to such a low temperature immediately after fertilization or when the eggs are in the process of early segmentation they suffer severely.

Thus in one experiment the eggs of *Fundulus* (fertilized with sperm of their own species) were distributed into a number of Erlenmeyer flasks and put on ice immediately after fertilization and kept at a temperature of between 0 and 2° C. After four hours, seven and a half hours, twenty-three hours, thirty-two hours, forty-eight hours, sixty-four, and ninety-six hours one lot was returned to room temperature. All the eggs taken out after forty-eight hours or later were dead; few of those taken out after thirty-two and twenty-three hours survived. A considerable percentage were abnormal and resembled the heterogeneous hybrids between *heteroclitus* ♀ and *Menidia* ♂. Most of those taken out after four and seven and a half hours survived, but about 20 per cent. to 30 per cent. were abnormal. Among the abnormal embryos a number of blind fish were found.

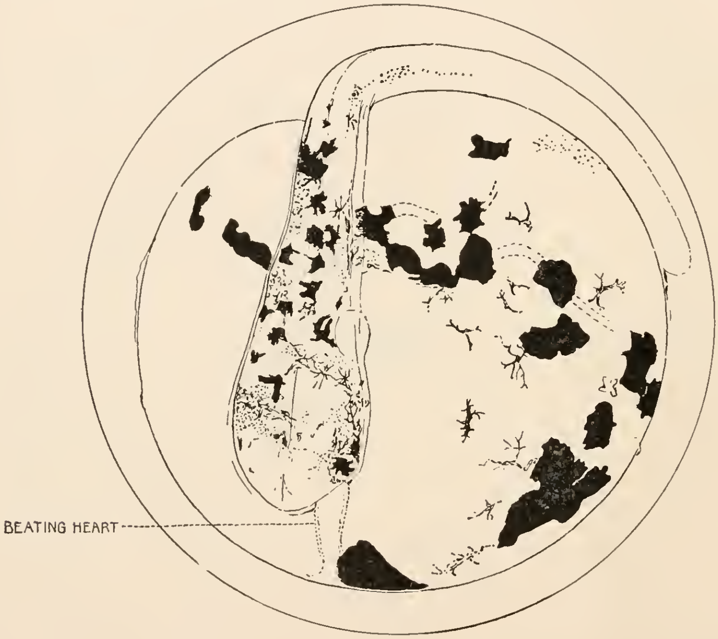

FIG. 9.

A second lot of eggs were put on ice four and a half hours after fertilization when they were in the 4-cell stage or beyond. These eggs were just as sensitive to the effect of the low temperature as those put on ice immediately after fertilization.

This experiment was repeated four times with the same result. In another experiment the eggs were allowed to develop to about the 128-cell stage at normal temperature before they were put into a temperature of 0° (about fifteen hours after fertilization). Eggs that were kept at that low temperature for two days were still able to develop into normal embryos but those that had been kept three days at the temperature of from 0 to 2° C. were practically all dead.

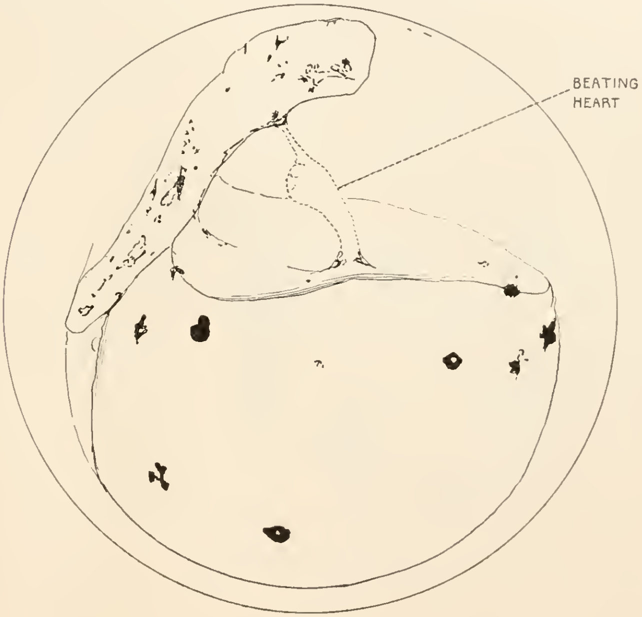

FIG. 10.

When the eggs were put into a temperature of from 0 to 2° C., after the embryo was formed and the circulation established, they could resist the low temperature for weeks. When put back to normal temperature they recovered and developed normally.

Further experiments are required to ascertain more accurately when the eggs become immune to the temperature of from 0° to 2°.

It is only this very low temperature at or very near the freezing point which is injurious for the newly fertilized eggs. If the temperature is a little higher, *e. g.*, 7° C., the newly fertilized eggs can live for weeks in it without being injured. Thus in the experiment just mentioned some of the eggs were put immediately after fertilization into a temperature of about 7° C. These eggs developed very slowly but no abnormal embryos were observed although some of the eggs were kept at a temperature of 7° C. for four weeks. Those that were kept still longer at that temperature suffered but probably not from the low temperature but from the fact that the flasks in which they were kept were closed.

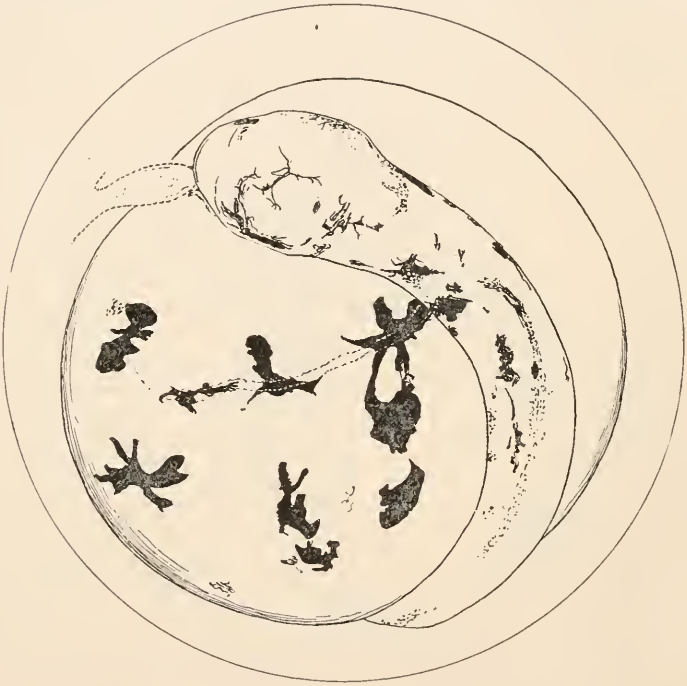

FIG. 11.

These experiments thus establish the interesting fact that immediately after fertilization the eggs of *Fundulus* are rapidly injured or killed when exposed to a temperature of 0° or a little above, while after the embryo is formed they can be exposed to such a temperature for a month without suffering. The newly fertilized eggs can, however, be exposed without injury to a temperature of 7° for weeks and possibly longer.

As already stated, among those eggs which were put into a temperature of from 0 to 2° C. a certain number had defective eyes. Thus the egg Fig. 10 was put immediately after fertilization into a temperature of between 0 and 2° for twenty-four hours and then put back to room temperature. The egg developed like a typical heterogeneous hybrid. Fig. 10 represents the embryo when two weeks old; it had a beating heart but no circulation. No eyes are noticeable. Fig. 11 represents an embryo from another experiment; in this too the egg had been kept for four hours (immediately after fertilization) on ice (0 to 2°). Apparently no eyes are formed but the circulation is established.

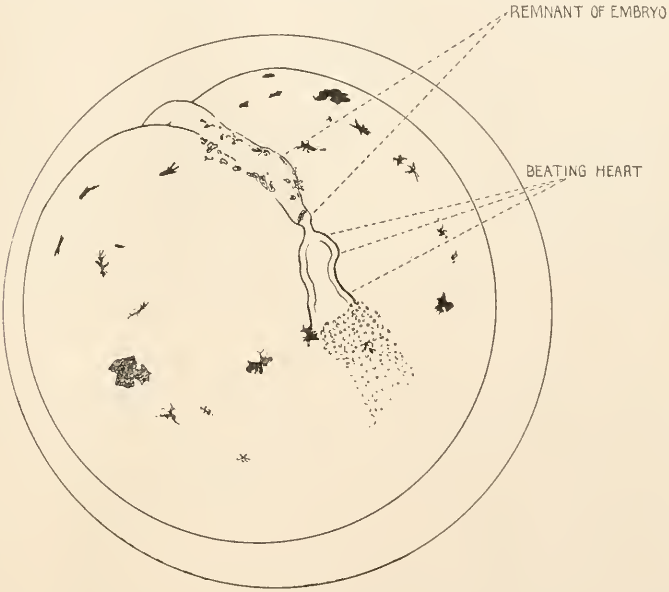

FIG. 12.

Figs. 12 and 13 are added to show to what extent the abnormal embryos, produced by a short exposure of the egg to a very low temperature (0°) immediately after fertilization, resemble the monstrosities which are formed in the case of heterogeneous hybridization. The embryo in Fig. 12 consisted practically only in a pulsating heart, what was left of the embryo was much less conspicuous than it is in the drawing. The embryo was ten days old. The egg had been put into the temperature of between 0 and 2° when it was in the 2-cell stage and had remained there for twenty-four hours.

The remnants of the embryo Fig. 13 had the same history. In this case, curiously enough, one eye with a lens and the tail was all that could be recognized. The monstrosity when drawn was eleven days old. An inexperienced observer might easily have concluded that in this case only the eye had been developed, while in reality the whole embryo had developed but the eye had survived while other parts perished.

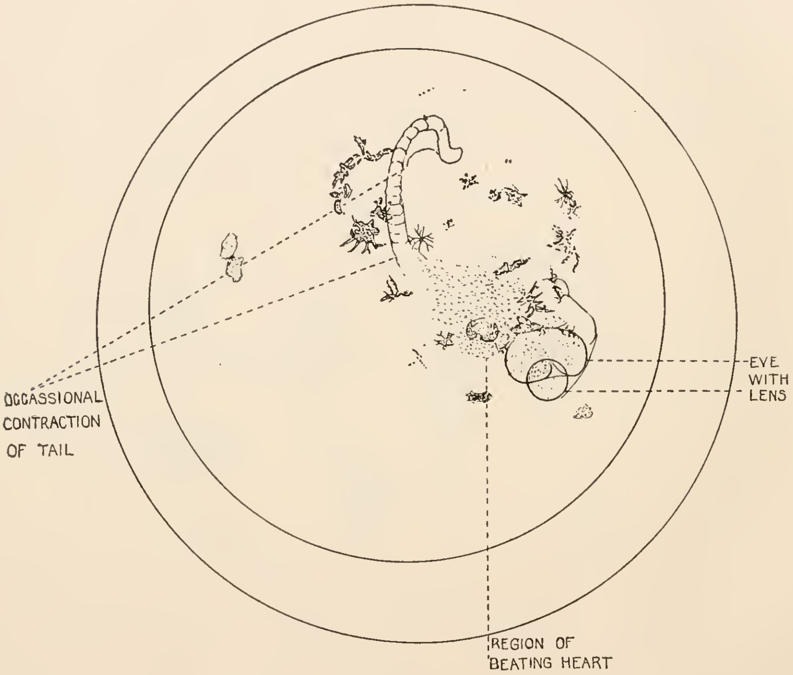

FIG. 13.

The analogy with teratomata is obvious.

V.

The writer was anxious to know whether it was possible to produce deficiencies in the eyes of *Fundulus* by raising them in the dark. It was necessary to carry on such an experiment with a large number of embryos. Since it was possible that a short exposure of the unfertilized egg or of the sperm to the light might already have some effect, the females and males of *Fundulus heteroclitus* to be used for the experiment were put into an absolutely dark room where everything was prepared for the experiment. The females and males were stripped of their sexual cells in the dark and the jars containing both sperm and eggs were put into dark boxes and kept in the dark room for four weeks. Although many embryos had died, hundreds had survived. All had perfectly normal eyes. This experiment confirms similar experiments made by the writer in previous years.

All these experiments show that while it is comparatively easy to produce blind *Fundulus* embryos, or, more correctly speaking, fish with degenerated eyes, by heterogeneous hybridization or by low temperature or by lack of oxygen (or by an excess of magnesium salts or by alcohol, as shown by Stockard and McClendon) no such result can be produced by lack of light.

VI.

If we consider all the facts in the case there is nothing at present to warrant the assumption that the blind cave animals owe the deficient development of their eyes to the lack of light, since lack of light is according to our present knowledge a less efficient agency in the causation of an abnormal development of the eye than a number of other injurious influences. Under these conditions we must be prepared to consider the possibility that many if not all the blind species found in caves owe their blindness to other influences than those of the cave. Eigenmann states that no blood vessels enter the eye of the blind cave salamander *Typhlotriton*. Since the experiments of Uhlenhuth as well as those of Stockard and of the writer reported in this paper indicate the importance of the circulation and of chemical factors in the development of the eyes it is not impossible that in the blind fish (Amblyopsidæ) as well as in the blind salamanders a hereditary disturbance in the circulation or nutrition of the eye or its surroundings is the cause of the degeneration of the eyes. There remains then the one difficulty mentioned in the beginning, namely to account for the fact that the relative number of blind species is greater in caves than in the open.

Eigenmann has shown that all those forms which live in caves were adapted to life in the dark before they entered the cave. These animals are all negatively heliotropic and positively stereotropic and with these tropisms they would be forced to enter the cave whenever they are put at the entrance to the cave. Even those among the Amblyopsidæ which live in the open have those tropisms of the cave dweller. This eliminates the idea that the cave adapted the animals for the life in the dark.

Eigenmann's observation makes it also clear that blind animals are comparatively rare in the open and that animals with normal eyes are not in the majority in the caves. Only those animals can thrive in the caves which for their feeding and mating do not depend upon visual mechanisms; and conversely animals which are not provided with visual mechanisms can only under exceptional conditions hold their own in the open where they meet the competition of animals which can see. This would account for the fact that in caves blind species are comparatively more prevalent than in the open.

In spite of all this, Eigenmann is inclined to assume that the darkness of the caves was a factor in promoting the blindness of the cave fauna. While those Amblyopsidæ which live in the open have already abnormal eyes those species of the family which are found in the caves have more degenerated eyes than those in the open and Eigenmann is inclined to ascribe this fact to an accumulated influence of the darkness. This would, however, compel us to account in a similar way for the incipient degeneracy of the eyes of those Amblyopsidæ that have never been found in caves and for the complete blindness of *Typhlogobius;* and would leave us at a loss to account for the presence of salamanders with perfectly normal eyes in the caves. It seems to the writer that consistency would demand to consider a common mode of origin of all these blind forms, namely as mutations, *i. e.*, as the result of some factorial change in the germ the cause and nature of which we are not yet able to define. Among the

Amblyopsidæ various mutations in the state of the eye may be constantly arising (as in the case of *Drosophila*); and some of these mutants, especially those that are perfectly blind, can not hold their own in the open while in the caves they can preserve and perpetuate themselves. This assumption would not exclude the possibility that Kammerer's observation may have been correctly interpreted by him, it would only provide for the possibility that his conclusion can not be generalized—a possibility which from the experiments mentioned in this paper must be seriously considered.

SUMMARY.

1. It is shown that blind embryos or more correctly embryos with degenerated eyes can be produced by heterogeneous hybridization in fish embryos (*e. g.*, *Fundulus heteroclitus* ♀ and *Menidia* ♂). Since in these cases as a rule no circulation exists the inference is possible that the anomalous condition of the eye may be due to lack of circulation.

2. Blind embryos of the pure breed of *Fundulus heteroclitus* may be produced by the addition of KCN to the sea water.

3. It is shown that immediately after fertilization (by sperm of their own species) and during the early stages of segmentation the egg of *Fundulus heteroclitus* is rapidly killed or injured if it is exposed to a constant temperature of about 0° (or slightly above); while it may be exposed to a slightly higher temperature (*e. g.*, 7° C.) for weeks without being injured. If the egg is exposed to the low temperature after the embryo is once formed it can resist the low temperature of from 0 to 2° C. for weeks without permanent injurious effects.

4. If eggs of *Fundulus heteroclitus* are fertilized with the sperm of the same species and exposed immediately after fertilization for a number of hours or a day to a temperature of between 0 and 2° C. abnormal embryos can be produced a certain percentage of which may show degenerated eyes.

5. Lack of light does not influence the development of the eyes of *Fundulus*.

6. It is pointed out that internal mutational changes and not lack of light may account for the blindness of certain cave fish and salamanders.

THE ABSORPTION OF FAT BY FRESHWATER MUSSELS.[1]

E. P. CHURCHILL, JR.

INTRODUCTION.

The present paper embodies the results of the first of a series of investigations designed to ascertain whether or not animals living in the water use food which is in solution in the surrounding medium in addition to "formed" food such as plankton, etc. If it is found that such food in solution is used, it is of importance to learn whether it is absorbed by the alimentary tract alone or to some extent by the epithelium of the outer body walls, especially that of the gills in the case of mussels.

Pütter ('08) was the first to advance the theory that food could be so taken. He based his conclusions in part on a comparison of the amount of carbon necessary for the maintenance of the organism with the amount furnished by the plankton. Pütter considered the latter too small for the needs of the animal and argued that it must use some carbon which is in solution in the water, resulting from the decay and disintegration of organic life. He further stated that the amount of material found in the alimentary canal is never large enough to supply the requisite quantity of carbon. Besides the alimentary canal, the uncutinized epithelium of the outer surface of the body, especially that of the gills, was thought to function in absorbing dissolved food. This process went on in addition of course to the digestion of "formed" food by the alimentary canal. Pütter concluded that the above conceptions applied to Protozoa, Porifera, Echinoderms, Crustaceans, Mollusks and Fishes. He tested the matter experimentally in two ways: first by noting that goldfish and perch lived much longer in solutions of asparagin, somatose and glycerin than in tap water: secondly by comparing the amount of oxygen needed to oxidize the lost weight of tissues of actinians, tunicates and fish while kept in their natural medium with the amount of oxygen actually used. As the latter was found to be greater than the estimated quantity needed he concluded that the extra oxygen was used in oxidizing some food that had been taken from the water where it was present in the form of a solute.

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[1] Contribution from the United States Biological Station, Fairport, Iowa. (Two of the experiments were carried out at the zoölogical laboratory of the Johns Hopkins University.) Published by permission of the Commissioner of Fisheries.

Knorrich (10), working with *Daphnia*, which lived 14 days in sterilized hay solution, concluded that nutriment was absorbed from the solution.

Kerb (10) kept eels in sugar solution and noted no diminution of the amount of sugar from day to day. He obtained similar results while working with *Corethra* larvæ in sugar solutions. Also he found that *Daphnia* lost in dry body weight as rapidly in solutions of peptones as in tap water.

Wolff (10), working with *Simocephalus*, found that it lived twice as long in bacteria-free water, which contained some dissolved carbon compounds, as it did in tap water. He made no observations as to body weight lost or gained.

Lipschütz (13) reviewed the entire subject including his own previously published experiments along that line and offered criticism of Pütter's work. Lipschütz noted that fish and eels when kept in nutrient solutions lost as much weight as in tap water. He also thought that Pütter overestimated the amount of material in solution in the water and underestimated the carbon content of the plankton. His general conclusions are the opposite of those of Pütter.

Lund[1] found that if Protozoa are kept in a weak soap solution they will absorb fat from such solution through their body walls. At his suggestion the fats used in the following experiments were rendered soluble in water by saponification.

I am especially indebted to Dr. Caswell Grave, at whose suggestion the work was undertaken, for his advice and aid on many occasions and his supervision of the preparation of the manuscript. I also desire to express my obligations to Dr. E. J. Lund for suggestions concerning some of the chemical reactions discussed in the latter part of this paper; to Dr. G. L. Houser for the use of several books and a microscope from the laboratories of the University of Iowa; and to Dr. R. E. Coker for aid rendered while the work was being carried on at the Fairport Laboratory.

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[1] Dr. Lund's paper is not yet published, his results having been communicated to me verbally.

Materials and Methods.

The mussels upon which the investigations were carried out were individuals selected from the more common species found in the Mississippi River near Fairport, Iowa. Both adult and juvenile specimens were employed. Care was exercised to choose for the experiments non-gravid mussels which were in a seemingly healthy condition, "shoulder-raked" or hand collected individuals being generally used in preference to those dragged out of the water by "crow-foot" hooks.

The water used in the experiments was that of the general supply for the Fairport Laboratory which had come from the Mississippi River through a filter which removed the mud and at least the greater part of the living animal and plant organisms.

The fats employed were those of olive oil and cottonseed oil. The results were the same in both cases as far as could be observed. The oils were saponified by boiling with sodium hydroxide, the resulting solution being diluted to strengths varying from .001 per cent. to .005 per cent. in different cases. This method can be used only with freshwater animals as the soap is precipitated from the solution in salt water.

The adult mussels experimented with were kept in glass aquaria containing about 5,000 c.c. of the fat solution of the desired strength. The bottoms of the aquaria were covered to a depth of an inch or two with coarse sand. Control individuals were run side by side with the experiments in similar aquaria containing filtered water only. The juvenile mussels were generally kept in smaller aquaria. In the cases of experiments extending over more than twenty-four hours, the solutions were changed daily, no effort being made to balance the aquaria. As the mussels from the river are accustomed to a current of water this seemed to be the closest approximation to natural conditions which it was possible to obtain in the laboratory.

The tissues of various portions of the adult animals experimented with and of the control mussels were sectioned by the freezing method, stained with Sudan III. and mounted in glycerin. Also sections of the tissues of the adult and of the entire bodies of the juvenile mussels were made after fixation in osmic acid and Muller's fixing fluid. In this method the higher alcohols were avoided by clearing the sections in clove oil from 85 per cent. or 90 per cent. alcohol and infiltrating in paraffin from the clove oil. The paraffin was removed with xylol and the sections were mounted in xylol balsam. Such sections were studied very shortly after mounting, as the fat is gradually dissolved out by the xylol.

In some cases the fat solution was stained by dissolving Sudan III. in the same to the point of saturation. The external appearance of various parts of mussels which had been kept in such stained solutions was noted. Also frozen sections of such mussels were examined to ascertain whether or not stained fat had been absorbed from the solution. In such cases the sections were mounted in glycerin directly after cutting.

Observations.

The mussels were found to live readily in .001 per cent. to .005 per cent. fat solutions. The only abnormal manifestation to be observed was that a considerable quantity of mucus was thrown off. Six adult individuals which were kept in fat solution for one month were living at the close of that period. At the expiration of the same length of time one of the control mussels of this experiment had died. After the adoption of the method of manipulating the aquaria outlined above, no mussels died while kept in fat solutions varying in strength from .001 per cent. to .005 per cent. However, beyond the fact that mussels can live for at least one month in fat solutions of these strengths, no evidence was secured concerning the relative longevity of those kept in fat solutions and of the control individuals. No weights were taken. Experiments calculated to ascertain whether or not the absorption of fat from the solutions employed is of advantage to mussels were deferred until the actual entrance of the fat into the tissues of the animal was proved. Emphasis was therefore laid upon the histological evidence of the absorption of fat and it is such evidence that this paper is designed to set forth.

Mussels which had been kept in unstained fat solution will be considered first. Camera lucida drawings were made of typical portions of the sections, care being taken to indicate as far as possible the number and position of the fat droplets.[1] In some cases the fat was so massed together that individual droplets were indistinguishable. Fat is represented in all the drawings by heavy black dots or areas.

The case of an adult specimen of *Quadrula ebena* which had been kept in .001 per cent. fat solution for 15 days will first be discussed. Sections of the gill filaments of this mussel were prepared after fixation in a solution of osmic acid and Muller's fixative. In such sections an abundance of fat droplets was to be seen in the epithelial cells and a number in corpuscles in the blood vessel in the interior of each filament (Fig. 1, Pl. I.). Very few fat droplets were found in sections of the control individual prepared by the same method (Fig. 2, Pl. I.). Frozen sections, after having been stained with Sudan III., gave results parallel to those just stated—a large number of fat droplets in the gill filaments of the mussels which had been kept in fat solution for 15 days (Fig. 3, Pl. I.), but practically nothing that took the Sudan III. stain in the control individuals. The epithelial lining of the water tubes of the gills of the individuals which had been kept in fat solution was also quite crowded with fat. In sections of the intestine of mussels which had been kept in fat solution, fat droplets appeared in the epithelial lining. Also in sections of palps and mantle fat droplets were revealed but not in such abundance as was true in the case of the gills. Fat droplets were quite numerous in the cells of the side of the mantle next to the body but there were practically none in the cells of the side lining the shell. The tissues of three adult mussels which had been kept in fat solution for 15 days and of two control individuals which had remained in filtered water for the same period were examined by the two methods of preparing sections. The former all presented abundant evidence of the presence of fat. The difference between those which had been kept in the solutions and the control mussels was as striking as that shown in Figs. 1 and 2.

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[1] The term "droplets" will in this paper be applied to the spherules of fat found in the tissues of the mussels which were studied. These droplets usually were of diameters varying from 5 to 10 microns, though in frozen sections of the gills instances were found in which the diameter was as great as 20 microns.

The best results were obtained from the study of juvenile mussels as in these cases the entire animal could be sectioned serially and the distribution of the absorbed fat over the entire body observed. A specimen of *Anodonta imbecillis* which had been kept in .002 per cent. fat solution for 10 days and a control specimen of the same species which had been kept in filtered water for the same length of time were selected as typical cases for study.

Figs. 4 and 5, Pl. II., showing corresponding parts of the foot, give an idea of the abundance and distribution of fat in the mussel which had been kept in the fat solution and of the almost entire absence of it in the case of the control specimen. (In this case as well as in that of several of the other figures, no effort has been made to draw the outlines of the epithelial cells, the intention being merely to represent the relative amount and distribution of the fat.) It will be noted that the fat is very abundant in the epithelium. It is present in some quantity in the interior of the folds of the foot and also within the deeper parts of the foot, apparently adhering to muscle fibers. The fat droplets exhibit some tendency here, and more markedly elsewhere, to gather together in clumps or to form chains. Several blood corpuscles which contain fat may be seen.

Fig. 6, Pl. II., shows a portion of the foot of the same individual, extending from the base of a fold to the blood vessel in the center of the foot. An abundance of fat was observable in the epithelium and quite numerous droplets appeared clinging to muscle fibers or enclosed within corpuscles in the central part of the foot.

Fig. 7, Pl. II., representing a portion of a fold of the foot, was drawn under the 1/12 oil immersion lens, an effort being made to depict the exact number and position and also the relative size of the fat droplets, muscle fibers and cells.

Fig. 8, Pl. I., shows in (*a*) the cross section of a liver tubule of the mussel used as the control, the few coarse stipples representing the fat present; and in (*b*) the cross section of the corresponding part of the mussel which had been kept in the fat solution. In the latter the liver cells are seen to be heavily loaded with fat, in some cases practically filled with it. A very great abundance of fat was found throughout the liver cells in all sections of this mussel.

Figs. 9, 10, and 11, Pl. I., are drawings made from the sections of the epithelial cells, respectively, of the intestine, of a gill filament and of the side of the mantle next to the body, of the specimen of *A. imbecillis* which had been kept in the fat solution for 10 days. They represent as accurately as could be drawn with the camera lucida the relative amount and arrangement of the fat droplets. Many corpuscles containing fat were found in the blood lacunæ immediately outside of the cells of the intestine. Some of these corpuscles were lying at the bases of the cells as shown in Fig. 9. Corpuscles containing fat were also found in nearly all blood vessels of the gill filaments. Many such corpuscles were in the position shown in Fig. 10, that is, close against the bases of the cells of the filament. In the mantle fewer corpuscles were seen. Here the fat was found adhering to muscle fibers and strands of connective tissue.

Figs. 12 and 13, Pl. III., represent mesenchyme cells and blood corpuscles of the mussel which had been kept in the fat solution and of the control individual respectively. In these sections may be observed the relation of the fat droplets to the tissues of the areas occupied by mesenchyme cells, which are especially numerous in the dorsal part of the body of the mussel. Fat was found scattered quite thickly throughout such regions in the case of the animal which had been in the solution.

The gill of an adult specimen of *Quadrula ebena* was sectioned immediately after it had been taken from the river. Fig. 14, Pl. I., represents the relative amount of fat found in its gill filaments. Somewhat more fat appeared in these sections than in those prepared from the tissues of the control mussels which had been kept in filtered water for 10 or 15 days but a very much smaller quantity was found than in the mussels which had been kept in fat solution.

The mussels which had been kept in the fat solution that had been stained with Sudan III. will now be considered. Before beginning the experiment the valves of the shell were opened sufficiently to permit the observation to be made that the tissues of the animal to be experimented with were of normal color and condition. The mussels were then kept in stained fat solution for periods varying from 5 to 15 days. At the expiration of these periods the gills of the adult and the gills, mantles and foot of the juvenile animals were found to be red or pink in color. Sections of the gills of such adult mussels were prepared by the freezing method. In these sections pink colored fat droplets were found within the epithelial cells of the gill filaments and of the water tubes (Fig. 15, Pl. I.). This fact furnishes additional proof of the absorption of fat from the solution. It renders negligible the consideration that the heavy loading of fat found in each case in the mussels which had been kept in the fat solutions might have been due to the chance use of an extraordinarily fat individual. Some of the juvenile mussels which had taken on a red color while they had been kept in the stained fat solution were then transferred to filtered water. The color remained visible in the foot and gills for more than a week in some cases. This would tend to show that the red colored fat was absorbed and oxidized within the organism and that the red color was not due merely to the adherence of the stained solution to the surface of the gill or foot.

In all, including those mussels which were kept in stained and unstained fat solutions and the control individuals, the tissues of twelve mussels were examined. The sections all revealed much fat within the tissues of the mussels which had been kept in the fat solutions and very little or none in those of the control individuals. Consideration of the above results leaves no doubt of the fact that fat may pass from a fat solution into the body of the mussel. Now there remains the question of whether it is all absorbed through the intestine or partly through the tissues of the outer body walls. Experiments intended to throw light upon this question were undertaken with juvenile mussels. These had been kept in water containing little food for several weeks so that their tissues contained little stored fat at the beginning of the experiment.

Four mussels were kept in a .005 per cent. fat solution for the several periods of 4, 7, 18 and 24 hours. A small amount of fat was found in the epithelium of the intestine, mantle, gills and foot of the individual of the 4-hour period. None was found in the other parts of the body. About the same amount and distribution of fat was observable in the case of the mussel which had been in the solution for 7 hours. In the animal which was kept in the fat solution for 18 hours a very appreciable quantity of fat was found in regions corresponding to those in which it had been found in the mussels which had remained in the solution for 4 and 7 hours. A small amount was noted in the tissues immediately beneath the epithelium. None was found in the deeper body tissues, a fact which is in striking contrast to the case of the mussel which had been kept in fat solution for 10 days. The mussel which had remained in fat solution for 24 hours contained a considerable amount of fat in the same parts of the body in which it was found in the cases of the individuals which had remained in the solution for 4, 7, and 18 hours. Even more fat was found in the epithelium of the gills, mantle and foot than in that of the intestine (Figs. 16, 17, 18, 19 and 20, Pl. III.). A very small quantity of fat was observable in the liver cells. In certain parts of the foot a moderate amount of fat appeared immediately beneath the epithelium, some of which fat was adhering to the muscle fibers. The fat droplets became progressively less numerous as the tissues were studied to a greater depth within the foot and none were found among the muscle fibers or in the other tissues making up the main central mass of the foot (Fig. 17, Pl. III.). The corpuscles, especially those found in the gills, contained fat in many cases. In the gills the crowding of the corpuscles against the bases of the cells of the filaments was marked (Figs. 18 and 19). Every appearance was given that the fat was absorbed from the solution by the epithelial cells of the gills and was taken up from them by the corpuscles in some cases and in others thrown directly into the plasma of the blood. Fig. 20, Pl. III., shows a portion of the epithelium of the mantle. Fat was seen in the epithelial cells of the side next to the body and for a short distance beneath them but it was not found scattered throughout the deeper parts of the mantle. No fat was found in the cells of the side of the mantle next to the shell.

The observations made on the mussels which were kept in fat solution for the periods of 4, 7, 18 and 24 hours may be summarized as follows:

1. More fat was found in the epithelium of the mantle, gills and foot than in that of the intestine.

2. In the case of the mussels which were kept in fat solution for 4 and 7 hours, fat was found only in the various epithelia.

3. In the mussel which remained in fat solution for 18 hours, much more fat than in the two above cases was found in the epithelium and a small amount was observable at various points beneath the epithelium, but none could be discerned distributed throughout the deeper body tissues.

4. In the mussel which had remained in fat solution for 24 hours, a very marked quantity of fat was found in the epithelium and a considerable amount appeared beneath the epithelium, diminishing in quantity, however, toward the interior of the body so that there was none distributed throughout the deeper body tissues.

5. No fat could be seen in the epithelium of the side of the mantle next to the shell but an abundance was present on the side which had been in contact with the solution.

6. Scarcely any fat was found in the cells of the liver.

From these facts it is probable that fat is absorbed by the outer epithelium of the body as well as by that of the intestine. Unless such were the case it would be very difficult to account for the presence of as much fat in the epithelium of the gills, mantle and foot as in that of the intestine, taking into consideration the fact that the mussels had been kept in the fat solution for such short periods, viz., intervals varying from 4 to 24 hours. It would also be difficult to account for the fact that such a very small amount of fat appeared within the deeper body tissues of these mussels while a very marked quantity was found in the epithelium of the outer body walls. It is very unlikely that the blood carried such a large amount of fat from the intestine to the outer epithelium in such a short time and during the same time carried none to the muscle fibers, mesenchyme cells or connective tissue or conveyed none to the liver, which is the normal fat storing organ. If all the fat were absorbed by the intestine it seems certain that the sections of one at least of the mussels which had been kept in the fat solution for the short periods would have revealed more fat within the deeper body tissues than was found there. Wherever fat was found beneath the outer epithelium of these mussels which had been in the solutions for the short periods, much the larger quantity lay in close proximity to that epithelium and the amount lessened to zero toward the interior of the body. While the quantity of fat beneath the epithelium increased with the length of the time the mussel was kept in the solution, in all cases it was greatest nearer the epithelium. Apparently the fat found lying closely beneath the outer epithelium of the body had been absorbed and passed into the deeper tissues by that epithelium and had not come from the intestine. Also, if all the fat were absorbed by the intestine and the blood did transport it almost entirely to the outer epithelium it would seem most likely that the cells of the side of the mantle next to the shell would receive at least an observable amount.

In order further to test the question of whether or not fat may be absorbed from a fat solution by the epithelial cells of the outer body walls of the mussel the following experiment was performed. The valves of three adult mussels were wedged open with bits of wood and the animals suspended on a wire rack over the fat solution so that only the ventral parts of the mantle and foot were immersed. The mouth and siphons were above the solution so that it was not likely that any of the solution could enter the intestine by way of the oral or anal openings. One mussel was thus treated for 6 hours, the other two for 22 hours. They were compared with a fourth individual which had not been in the solution, all four mussels having been removed from the river only four or five days previous to the experiment. One of the animals which had been treated as above for 22 hours did not have much fat throughout the deeper body tissues but a moderate amount appeared in the epithelium of the part of the mantle which had been immersed in the solution. The other mussel which had been treated for 22 hours contained some fat throughout the body and a heavy amount in the epithelium of the part of the mantle which had been exposed to the solution (Fig. 21*a*, Pl. III.). The control individual for this experiment contained much more fat throughout the deeper body tissues than the other three individuals but very little in the epithelium of the ventral part of the mantle (Fig. 21*b*, Pl. III.). The ventral part of the foot of the mussel which had been so treated for 6 hours revealed a moderate amount of fat in the epithelium but scarcely any in other parts of the foot. The fat found throughout the deeper body tissues of these individuals was of course previously stored fat, the mussels, as stated above, having been removed from the river but a short time. The point intended to be made is that a heavier loading of fat is found in the epithelium which had been in contact with the fat solution than in that which had not. While there is a possibility that some solution may have been carried by ciliary action up the mantle to the mouth, this probably did not occur as, in sections of the intestine, absolutely no fat was found in the epithelial cells. It seems highly probable that the epithelium of the mantle and foot absorbed the fat directly from the solution.

In regard to the mechanism of absorption of the fat by either the intestine or body walls little can be offered. The matter is quite complicated, some of the same possibilities entering here that serve to render the method of absorption of fat by the mammalian intestine as yet obscure. In some of the sections, especially in those cut by the freezing method, numerous droplets which took the stains used were found closely attached to the outer ends of the epithelial cells of the gills or mantle. These droplets were probably the fatty acids, a small part of which were present in the soap solution due to hydrolysis, by which process sodium hydroxide and the fatty acids would be formed; the remaining droplets were no doubt due to a slight acidity of the surface of the living cells resulting from the union of carbon dioxide from the cells with the water, forming carbonic acid. The fat may have been taken into the cells from these droplets upon the surface by phagocytic action or by solution in the plasma membrane, or it may have come directly from the sodium soap, the collection of the droplets upon the surface of the cells being a phenomenon extraneous to the process of absorption. The latter interpretation seems the more probable as the droplets were not found upon the surface of the cells in nearly all the sections of tissues which had absorbed fat. The sodium may enter the cells with the acid radical and later be separated, or the fatty acid may be split off outside and enter the cell alone. As the fatty acid radical is the constituent of the fat molecule which takes the stain, only that radical could be followed in my preparations. In nearly all cases a fairly definite, though narrow, area lies between the outer ends of the cells and the fat clusters within. This band is the plasma membrane. The fact that no fat droplets could be observed actually in this membrane points to the conclusion that absorption of fat is effected by its solution in the plasma membrane and precipitation within the cell.

The manner of transportation of the fat within the body deserves mention. The bases of the cells of the intestine are in contact with blood lacunæ. Blood vessels traverse the gill filaments. A meshwork of blood lacunæ lies in the connective tissue beneath the epithelium of the mantle and foot. A considerable amount of fat is taken up and transported by the corpuscles to various parts of the body. Evidence of this is clearest in sections of the intestine and gills (Figs. 1, 9, 10, 18 and 19), in which corpuscles containing fat are found in close contact with the bases of the epithelial cells. Fat-loaded corpuscles are found in the blood spaces throughout the body of the mussel which had been kept in fat solution for several days. However, sections of the foot and mantle reveal many fat droplets lying immediately beneath the epithelium and clinging to muscle fibers or to the connective tissue instead of being contained in corpuscles (Figs. 4, 7, 11 and 17). No doubt, besides being transported by corpuscles, fat is thrown directly into the blood stream and carried thus to various parts of the body. In some cases the fat droplets may have become attached to muscle fibers, etc., as was observed in the sections, during the preparation of the tissues. In other cases the attachment may have occurred before the mussel was killed, the tissues to which the fat droplets were adhering being those which were later to oxidize the fat.

SUMMARY.

1. Fat which is in solution in water can be absorbed by fresh-water mussels.

2. Such absorption is accomplished by the epithelium of the intestine and also most probably by that of the gills, mantle and foot.

3. Fat is transported both by the blood corpuscles and by the plasma directly.

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EXPLANATIONS OF PLATES.

All drawings were made with camera lucida; Leitz Oc. No. 4, Obj. No. 6, unless otherwise indicated. The drawings are of sections prepared by the osmic acid method unless otherwise stated. Fat is represented by heavy black dots or areas.

PLATE I.

FIG. 1. Gill filament of adult *Quadrula ebena* after remaining in fat solution 15 days. *a*, epithelial cells. *b*, blood vessel. *c*, blood corpuscles. *d*, chitinous rods.

FIG. 2. Gill filament of the control for above figure: mussel had remained in filtered water 15 days. *a*, *b*, *c*, *d*, same as in Fig. 1.

FIG. 3. Gill filament of adult *Quadrula ebena* after remaining in fat solution 15 days. Frozen section, Sudan III.

FIG. 8. *a*, Cross section of liver tubule of juvenile *A. imbecillis* after remaining in filtered water 10 days, the control. *b*, Cross section of liver tubule of juvenile *A. imbecillis* after remaining in fat solution 10 days.

FIG. 9. Epithelial cells of anterior part of intestine of juv. *A. imbecillis* after remaining in fat solution 10 days. *a*, epithelial cells. *b*, blood corpuscles.

FIG. 10. Gill filament of juv. *A. imbecillis* after remaining in fat solution 10 days. *a*, blood corpuscles.

FIG. 11. Portion of epithelium on side of mantle next to the body of juv. *A. imbecillis* after remaining in fat solution 10 days.

FIG. 14. Gill filaments of adult *Quadrula ebena* sectioned immediately after taken from the river.

FIG. 15. Portion of gill filament of adult *Quadrula ebena* after remaining in Sudan stained fat solution 15 days. Black areas represent droplets of pink colored fat. Frozen section.

Fig. 1

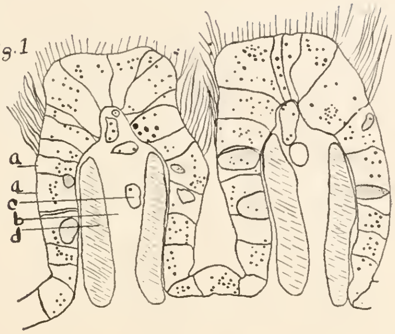

Fig. 2

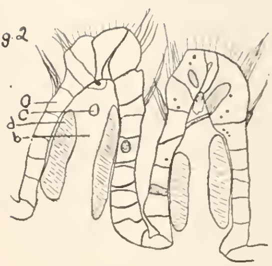

Fig. 3.

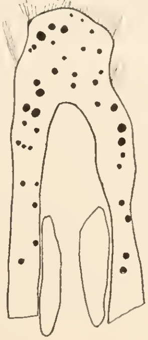

Fig. 14.

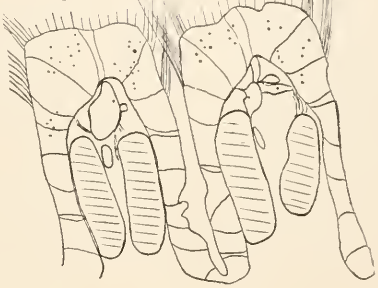

Fig. 9

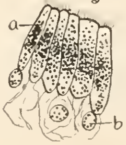

Fig. 11

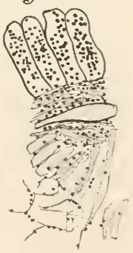

Fig. 8.

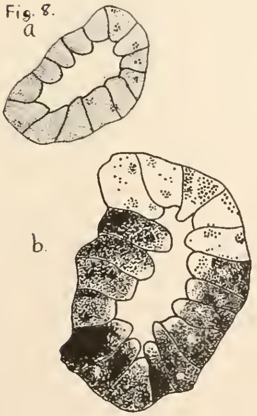

Fig. 10

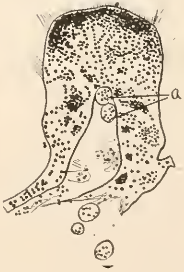

Fig. 15.

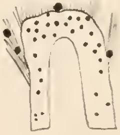

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PLATE II.

FIG. 4. Portion of foot of juv. *A. imbecillis* after remaining in fat solution 10 days. Epithelial cells not indicated. Fat shown heavily massed in epithelium and adhering to muscle fibers and in corpuscles. *a*, muscle fibers in cross section. *b*, blood corpuscles. *c*, muscle fibers in longitudinal section.

FIG. 5. Portion of juv. *A. imbecillis* after remaining in filtered water 10 days. Control for Fig. 4.

FIG. 6. Portion of foot of same individual as in Fig. 4. *a*, epithelium. *b*, muscle fibers in longitudinal section. *c*, muscle fibers in cross section. *d*, blood corpuscles.

FIG. 7. Portion of fold of foot of same individual as in Fig. 4. 1/12 oil immersion. *a*, epithelial cells. *b*, muscle fibers.

Fig. 7.

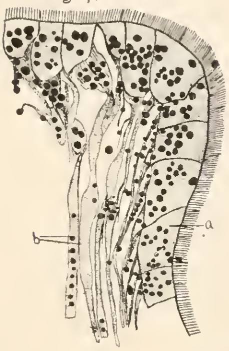

Fig. 4.

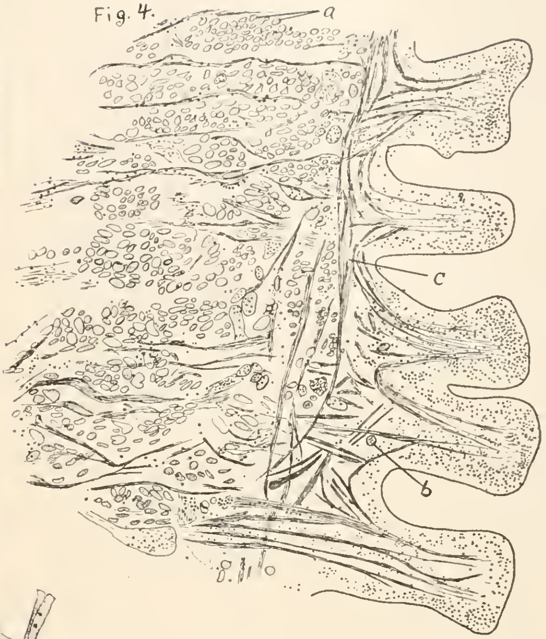

Fig. 6.

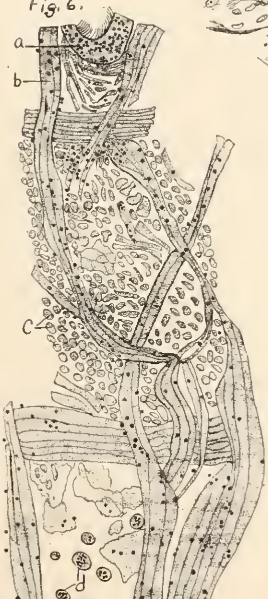

Fig. 5

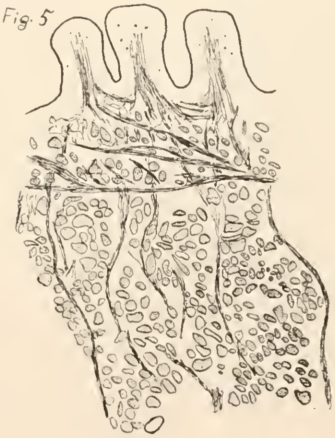

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PLATE III.

FIG. 12. *a*, mesenchyme cells and *b*, blood corpuscles of juv. *A. imbecillis* after remaining in fat solution 10 days.

FIG. 13 *a* and *b*. Mesenchyme cells and corpuscles of the control kept in filtered water.

FIG. 16. Epithelial cells of intestine of juv. *Q. pustulosa* after remaining in fat solution 24 hours.

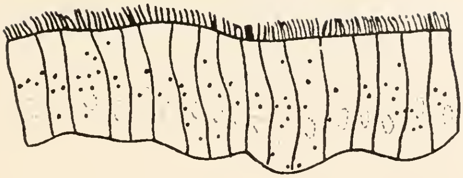
FIG. 17. Portion of foot of same individual as in Fig. 16.

FIGS. 18 AND 19. Gill filaments of same individual as in Fig. 16.

FIG. 20. Portion of side of mantle next to body of same individual as in Fig. 16.

FIG. 21. *a*. Epithelial cells of that portion of mantle immersed in fat solution 22 hours. The mouth of this mussel was kept above the solution. *b*. Epithelial cells of corresponding part of mantle of mussel used as control for Fig. 21 *a*.

Fig. 16.



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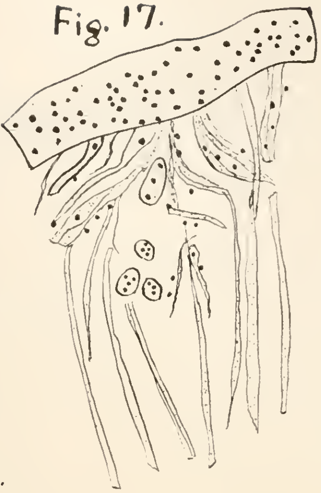


Fig. 12 a.

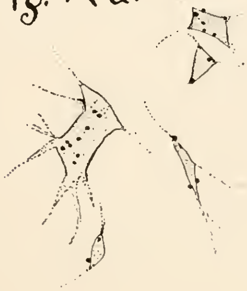


Fig. 12.b.

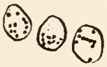


Fig. 13.b.

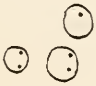


Fig. 21.a

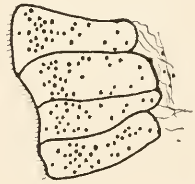


Fig. 13a.

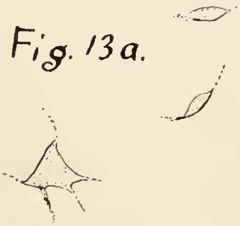


Fig. 18.

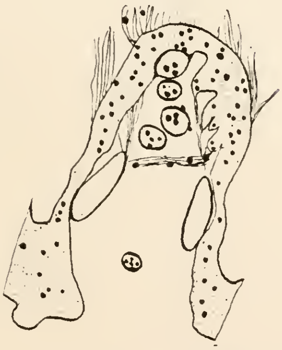


Fig. 21.b.

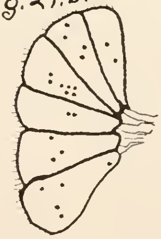


Fig. 19.

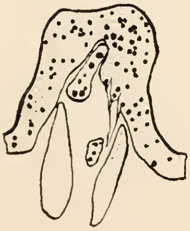
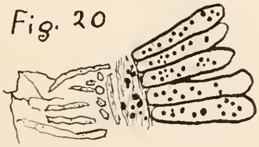


Fig. 20



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BIOLOGICAL BULLETIN

SEXUAL REACTIONS BETWEEN HERMAPHRODITIC AND DIŒCIOUS MUCORS.[1]

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Introduction.

Conjugation among the Mucors has been assumed to represent the simplest type of reproduction acknowledgedly sexual in character. Some even have denied the term sexual to the union of morphologically similar gametes such as occur in this group. The present article will present further evidence in favor of the author's contention that although the process is morphologically a simple one, conjugation in the Mucors is as definitely a sexual process as the morphologically more complex type of reproduction in higher forms and that the sexes seem even more sharply distinct.

It has been shown by the writer ('04, '09) that the majority of the forms among the Mucors are diœcious, with the sexes separated in male and female races which are capable of being propagated apparently to an indefinite number of vegetative generations by means of nonsexual spores formed in sporangia. In all the diœcious species carefully investigated the opposite gametes, which are produced and unite to form zygospores when the two sexual races of a given form are grown together, do not appear to differ morphologically. Lacking a definite criterion which an inequality of the gametes would have afforded, the writer has provisionally designated the opposite sexes in these forms by the signs (+) and (−) on account of a generally greater vegetative luxuriance of one sex over the other. That in reality the two sexes are represented in the (+) and (−) groups is shown by the sexual reaction which may occur not only when the (+) and (−) races of the same species are grown together and perfect zygospores are produced, but also by the sexual reaction which may occur when (+) and (−) races belonging to different species are grown together. This reaction between the opposite races of different species has been called "imperfect hybridization" since it does not lead to the formation of perfect hybrid zygospores, but usually stops short with the formation of progametes, though occasionally gametes are produced which, however, never unite.

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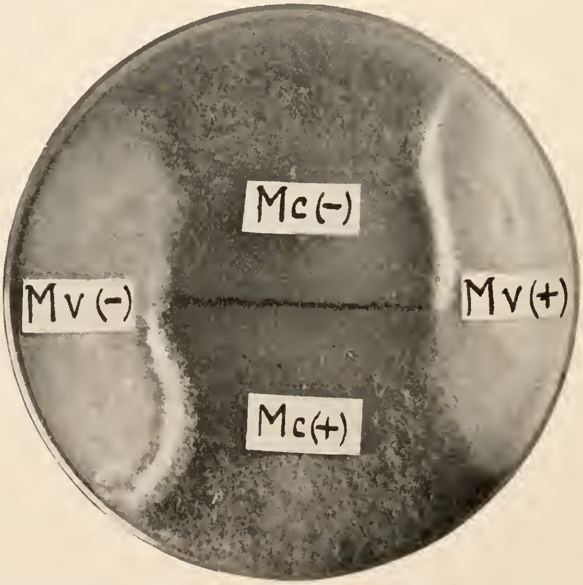
[1] Report of investigation carried on, 1912–13, at the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.

A sexual race of a diœcious species if grown between the (+) and (−) races of another test species used as a standard, will show a line of imperfect hybridization on one side only. Some of the hermaphroditic species, on the other hand, when similarly grown, show a response to both (+) and (−) test races and produce therefore two lines of imperfect hybridization. When



Petri dish culture showing dark line of zygospores between the (+) and (−) strains of a species of *Choanephora* (*MC*) and white lines of "imperfect hybridization" between the strains of this species and the opposite strains of *Mucor V.* (*MV*).

the sexual reaction is very strong it may become evident to the naked eye by the formation of a distinct white line as is shown in the adjoining photograph.

Some few species in the hermaphroditic group are distinctly heterogamic with a constant difference in size between the conjugating gametes. It seems reasonable to consider the larger gamete female and the smaller male. Upon this basis, if a sexual reaction could be established between these unequal gametes and the (+) and (−) races, the race reacting with the larger female gamete must be considered male, while the race reacting with the smaller male gamete must be considered female. This was pointed out in 1906 ('06*b*) and in a recent article ('13*b*) it has been shown in brief that sexual reactions have been induced between unisexual races and heterogamic species. The conclusion is reached that the (−) race is to be considered male and the (+) female. The present article will give the detailed results which have formed the basis for this conclusion.

HETEROGAMIC HERMAPHRODITES.

Since inequality in the size of the gametes in heterogamic species is used as the criterion of sex, it is essential that there be no doubt as to the process of sexual reproduction in these forms. With the exception of *Syncephalis*, which is difficult to cultivate, the heterogamic forms at present known are *Dicranophora fulva* and an undescribed American Dicranophora, neither of which are now in cultivation, *Absidia spinosa*, *Zygorhynchus heterogamus*, *Z. Moelleri* and a number of forms recently described (including *Z. Vuillemini*) which are perhaps too closely allied to *Z. Moelleri* to be deserving of separate names.

The writer in a recent publication ('13*a*) has made a restudy of the genus *Zygorhynchus* and attempted to correct certain misinterpretations of zygospore formation which had been advanced for this genus. Moreau ('12, '13) also, both before and since the publication mentioned, has insisted upon the correct interpretation. So far as concerns the formation and union of unequal gametes, the process is essentially similar to that in *Absidia spinosa* which is the heterogamic species most extensively used in the tests discussed in the present paper.

Absidia spinosa was first found by Lendner and is correctly described by him (Lendner '08). Its zygospores agree with other Absidias in possessing circinate outgrowths which develop from the suspensor soon after the formation of gametes. It differs from other Absidias, which it resembles in its method of nonsexual multiplication, by being hermaphroditic (cf. right-hand side of Fig. 8, Plate I.) and heterogamic (Fig. 4). Figs. 1–7 represent stages in development in living material drawn at the times indicated in the legend. A considerable number of conjugations have been followed in living material and stages have been studied in stained and mounted material. Unequal gametes, such as are shown in Fig. 4, unite, as in Fig. 6, and the zygote thus formed grows into a mature zygospore such as is shown in Fig. 7. In moist chamber cultures, drops of fluid generally accumulate around the uniting gametes (Fig. 6), often causing appearance of transverse lines that suggest additional cross walls. They can be distinguished from cross walls, however, by forcing the conjugating filaments against condensed moisture on the under side of the cover-glass. Perhaps the most typical form of conjugation is that shown in Fig. 8, where a rather stout branch on the right has applied itself to the more delicate termination of the main axis causing the production from the latter of a small male gamete, while it produces from its own enlarged tip a large female gamete. Curved outgrowths arise from the swollen suspensor but are not produced from the side of the smaller gamete. In *Zygorhynchus* a septum is regularly formed across the main axis just above the origin of the stout conjugative branch. This septum is as regularly absent in *Absidia spinosa*.

When for any reason the process of conjugation is arrested after the formation but before the union of gametes, these sexual cells may round themselves off, form thick walls and become azygospores. This is brought about in greater or less degree when the conjugating filaments in a moist chamber culture become immersed in a drop of condensed water on the underside of the cover-glass. Whatever the cause of the check to normal conjugation, the size of the azygospores depends upon the size of the gametes from which they develop. Thus in Fig. 33 it would be possible to label the azygospores of *A. spinosa* male and female, from their relative size alone, even if one were not subtended by a larger suspensor and surrounded by the outgrowths peculiar to the side of the female gamete. Similarly *Zygorhynchus* may produce male and female azygospores from adjacent gametes that have failed to fuse (Fig. 13). As will be shown later, azygospores may be produced from gametes that have formed as the result of a sexual reaction with a different species. If the opposite gametes of hermaphroditic forms are strictly male and female, it would theoretically be possible to transform a hermaphroditic species into male and female races by germinating its male and female azygospores. This has been attempted ('06*a*) and germinations have been obtained of the azygospores of *Sporodinia*. The mycelia that have thus been obtained produced only a very scanty growth and soon died out before it was possible to test their sexual reaction. Germinations of other azygospores, so far as the writer is aware, have never been critically investigated.

Homogamic Hermaphrodites.

Spinellus fusiger, *Sporodinia grandis*—two forms found upon fleshy fungi,—*Mortierella polycephala* and *Mucor Genevensis*—are the only homogamic forms definitely known among hermaphroditic Mucors. In none of these is there apparently a constant difference in the size of the gametes. Neither *Spinellus* (which is difficult to cultivate on artificial media) nor *Sporodinia* shows sexual reactions with other species. *Mortierella* has not been available for study. *M. Genevensis*, of which the writer has races from four distinct sources as well as derived mutants, shows a strong sexual reaction with both (+) and (−) sexes of diœcious species. The reaction has already been noted ('04*b*, p. 311, pl. IV., fig. 56). Observation on living material shows the reaction to be similar to imperfect hybridization between different diœcious species. The formation of azygospores, however, has been observed in different races of this hemaphroditic species as a result of the stimulus of contact with (+) and (−) strains of Mucor V. Azygospore formation will be further discussed under *Absidia spinosa* below.

Diœcious Species.

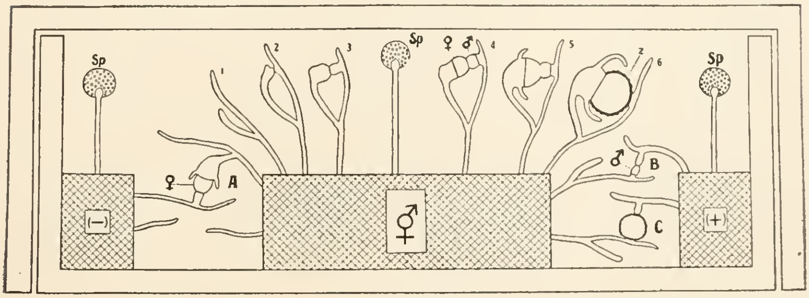
No diœcious form of the Mucors is known to be regularly heterogamic. In *Rhizopus* ('04*b*, Fig. 15) and in other diœcious species the writer has found that the inequality in size between conjugating gametes has no necessary relation to sex, since the larger gamete is formed sometimes by one sex, and sometimes by the other. In many species, however, the (+) race regularly shows a decidedly greater vegetative vigor than its (−) mate. This greater vigor of growth may be responsible for the fact that the conjugative filaments arising from the (+) mycelium average stouter than those from the (−), and this difference may be accompanied by a similar difference in the gametes and suspensors. One was generally able to recognize the (+) from the (−) sides of the line between the two sexes of Mucor V. by this greater vigor of the (+) conjugative filaments but even in this form where the distinction is most marked, the stouter of the conjugating filaments is not invariably on the (+) side. Mucor V. is a form found by the writer ('04*b*) in 1904. It is apparently not specifically distinct from *M. hiemalis* Wehmer, the sexual races of which were isolated by Hagem ('08) and forms zygospores, though in no great abundance, with the strains of this name sent from the "Centralstelle" at Amsterdam. It differed from the Amsterdam material, however, in its much greater sexual vigor. In 1912 when the reactions described in the present paper were investigated, the Amsterdam *M. hiemalis* failed to show any reactions with other species tested, while Mucor V. was sexually the most active of all the Mucors known. At the present writing, however, Mucor V. has apparently become weakened in sexual activity. Hagem ('08) reports a similar loss of sexual activity in one of his strains of *M. hiemalis*.

Reactions between Diœcious Species and Absidia spinosa.

The difficulties in technique involved in following the sexual reactions in a thicket of filaments have been overcome by growing the heterogamic hermaphrodite (☿) in a Petri dish between the (+) and (−) test strains and cutting channels in the nutrient agar between the different growths. If the Petri dish be then inverted, the growth of the reacting filaments may be followed in mid air in the channels, under the low power objective.

Absidia spinosa is the only heterogamic species which has been found to give reactions with both (+) and (−) races and Mucor V. is the only diœcious form that will react with both male and female gametes of *A. spinosa*. These have accordingly been most extensively employed in making the tests shown in the accompanying diagram.

In the left-hand channel at *A* in the diagram, a filament from the (−) race is shown giving a sexual reaction with the larger ♀ gamete of the hermaphrodite, while in the right-hand channel at *B* a filament of the (+) race is figured, showing a sexual reaction with the smaller ♂ gamete of the hermaphrodite. The



Diagrammatic representation of a Petri dish culture showing a heterogamic hermaphroditic mucor (☿) in the center separated by channels on either side from the (+) and (−) races, respectively, of a diœcious species.

Sp., Sporangia containing spores by means of which the plant may be reproduced nonsexually.

1–6, stages in development of a hermaphroditic zygospore from unequal male and female gametes.

A, sexual reaction between a (−) filament and female gamete.

B, sexual reaction between a (+) filament and male gamete.

C, a male azygospore formed at stimulus of contact with a (+) filament.

male gamete, which has been cut off from a filament of the hermaphrodite at the stimulus of contact with a (+) hypha, frequently surrounds itself with a thick wall and assumes the appearance of a resting azygospore, as is shown at *C*.

Figs. 20–24, Plate II., are drawings from living material. The female gamete of *A. spinosa* is recognized not only by its larger size but also by the curved outgrowths that arise from its suspensor. It will be observed further from these figures that the female gamete is formed from a rather stout branch which appears to have been attracted by Mucor V. (−) and drawn away from the delicate termination of the main axis with which it would normally have conjugated (cf. Fig. 8). In order to obtain such reactions as shown in Figs. 20–24, some care had to be exercised in regard to the condition of the culture. If the nutrient is too favorable for growth the filaments will choke the channels and cannot be followed. If the nutrient is deficient, conjugations will not occur. The depth and width of the channels and the time at which they are cut must be carefully regulated or no reactions can be observed. They have been obtained and studied in a sufficient number of cases, however, to leave no doubt as to their occurrence. Mucor V. is the only form whose (−) race was found to give a sexual reaction with *A. spinosa.*

The sexual reaction of *A. spinosa* with the (+) race of Mucor V. is entirely different from what has just been described. At the line of contact between the two mycelia, the filaments are much branched and imperfect hybridizations are abundant. Figs. 18 and 19 show simplifications of this condition from living material. Fig. 17 shows gametes cut off from *A. spinosa,* while Figs. 14–16 show such gametes transformed into dark thick-walled azygospores. Fig. 15, which shows the progamete of Mucor V. (+) rounded off and in bare contact with the azygospore of *A. spinosa,* is a typical condition.

The (+) race of *Absidia cærulea* (*A* (+), Figs. 8–10) shows a less active sexual reaction with *A. spinosa* but similar to that shown by Mucor V. (+), and the same is true of *A. cylindrospora* and Mucor N. The (+) strains of all four diœcious species mentioned are capable of stimulating the production of small male gametes from filaments of *A. spinosa* and their transformance into dark thick-walled azygospores. Unfortunately it has not yet been possible to bring these azygospores to germination. It would be interesting to discover if their germinations would give rise to unisexual male mycelia.

Judging from the reactions just described between *Absidia* *spinosa* and the sexual races of Mucor V., one would seem justified in considering the vegetatively more vigorous (+) race as female, and the less vigorous (−) race as male. The terms male and female therefore may be used hereafter in distinguishing the sexual races of diœcious Mucors.

REACTIONS BETWEEN DIŒCIOUS SPECIES AND ZYGORHYNCHUS.

Zygorhynchus Moelleri shows no sexual reaction with the (−) race of Mucor V. but contact with its (+) race causes the production of delicate, contorted, warty filaments which take part in imperfect hybridizations as shown in Fig. 12. The gametes formed by *Z. Moelleri* in contact with Mucor V. (+), frequently are transformed into azygospores shown in Fig. 11. *Z. Vuillemini* apparently is identical with *Z. Moelleri*, at least so far as its sexual reactions with Mucor V. are concerned.

In contrast with these two Zygorhynchus forms just discussed which show only a (−) or male tendency in contact with Mucor V., *Z. heterogamus* gives evidence of a female tendency. Its reactions with the (−) race of Mucor V. are shown in Figs. 25–28. It does not seem to react with (+) races and even with the (−) race it is not easy to induce reactions and those that result do not lead to azygospore formation. If Mucor V. (−) in Fig. 25 is actually showing a sexual reaction with the male gamete of *Z. heterogamus* as might appear from its position on the terminal filament of the latter, the evidence would be in conflict with that to be drawn from the reactions with *A. spinosa*, *i. e.*, that the (−) race is male and the (+) race female. It will not be possible to attach much significance to the individual reactions in *Zygorhynchus*, however, since so far no form of this genus has been induced to react with more than a single sexual strain of the test diœcious species.

Z. Vuillemini var. *agamus* is a zygosporeless strain which Namyslowski (10) separated from a culture of *Z. Vuillemini*. It was found impossible to induce zygospore formation from cultures of this species supplied by the Centralstelle. In contact with the (+) race of Mucor V. (Figs. 30–32) however, it reacted like the sexually active strain of *Z. Vuillemini* and even produced azygospores (Fig. 32). This form therefore has a male tendency.

Judged by their reaction with the (+) and (−) strains of Mucor V., the forms *Z. Moelleri*, *Z. Vuillemini* and *Z. Vuillemini agamus* have a male tendency, while *Z. heterogamus* has a female tendency. It is in harmony with this classification that *Z. Vuillemini agamus* produces azygospores in contact with *Z. heterogamus* (Fig. 29).

SUMMARY

It has been shown that sexual reactions called "imperfect hybridization" may occur when hermaphroditic species of Mucors are grown in contact with the sexual races of diœcious forms. Certain of these hermaphrodites are heterogamic, showing a constant difference in the size of their gametes. It is assumed that the larger gamete is female and the smaller one male. The race of diœcious Mucors provisionally called (+) shows a sexual reaction with the smaller or male gamete while the (−) or vegetatively less vigorous race shows a reaction with the larger or female gamete. It is concluded therefore that the (+) race of diœcious Mocurs is female and the (−) race, male.

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EXPLANATION OF PLATES.

All figures were outlined with the aid of a camera lucida. Those in outline were taken from stained and mounted material. Those stippled show development in living material from moist chamber cultures. Figs. 8–12, 18–24 and 31–32 were viewed with a low power objective and were magnified by the use of increased tube length or higher eye-pieces. Other figures represent high power drawings.

Plate I.

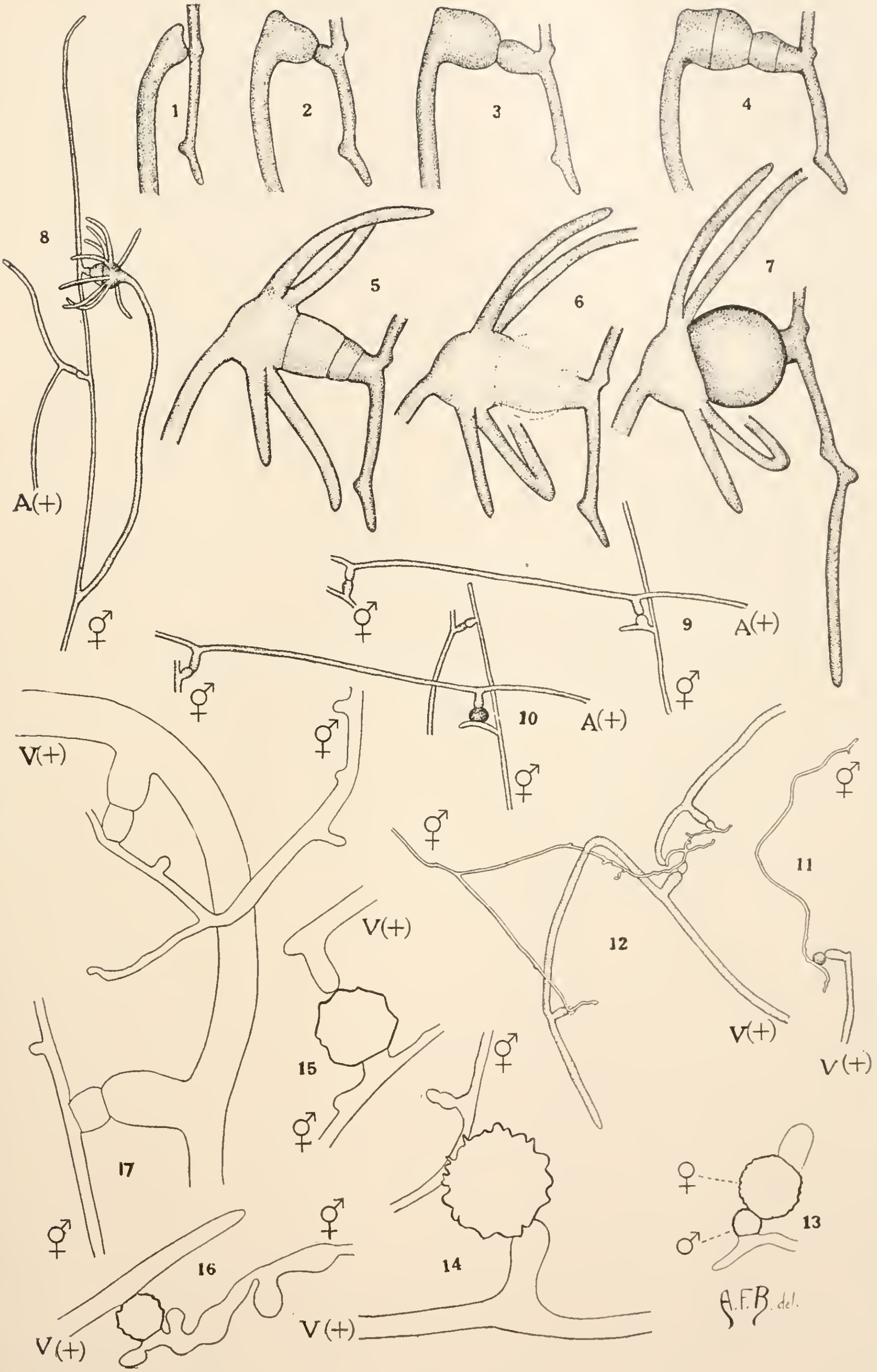
Figs. 1–7. *Absidia spinosa.* Consecutive stages in zygospore development from living material. Only those outgrowths in median focus are represented. Drawings were made at following hours: Fig. 1 at 4.15 P.M., Dec. 4; Fig. 2 at 4.52 P.M.; Fig. 3 at 5.50 P.M.; Fig. 4 at 7.10 P.M.; Fig. 5 at 12.30 A.M., Dec. 5; Fig. 6 at 2.15 A.M.; Fig. 7 at 3.00 P.M. The dotted circle in Fig. 6 represents the outline of a drop of fluid around the young zygote.

Figs. 8–10. "Imperfect hybridization" between *Absidia spinosa* (☿) and the (+) race of *Absidia cærulea* (*A*(+)). Fig. 8 on right, typical conjugation of *Absidia spinosa;* on left, "imperfect hybridization." Fig. 9 drawn at 8.30 A.M., Sept. 23. Fig. 10 drawn at 4.00 P.M., Sept. 24. Note azygospore formed from the male gamete of the ☿ hypha.

Figs. 11 and 12. "Imperfect hybridization" between *Zygorhynchus Moelleri* (☿) and *Mucor V.* (+). Fig. 11, male azygospore from ☿ hypha. Fig. 12, three "imperfect hybridizations."

Fig. 13. Azygospores formed from male and female gametes of *Zygorhynchus.*

Figs. 14–17. "Imperfect hybridizations" between *Mucor V.* (+) and *A. spinosa* (☿). Figs. 14–16, azygospores developed from male gametes of the ☿. Fig. 17, gametes on the ☿.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2
3
4
8
5
6
7
A(+)
⚥
⚥
9
A(+)
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⚥
10
A(+)
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V(+)
⚥
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11
12
V(+)
V(+)
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15
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17
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13
♀
♂
16
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14
V(+)
V(+)
A.F.B. del.

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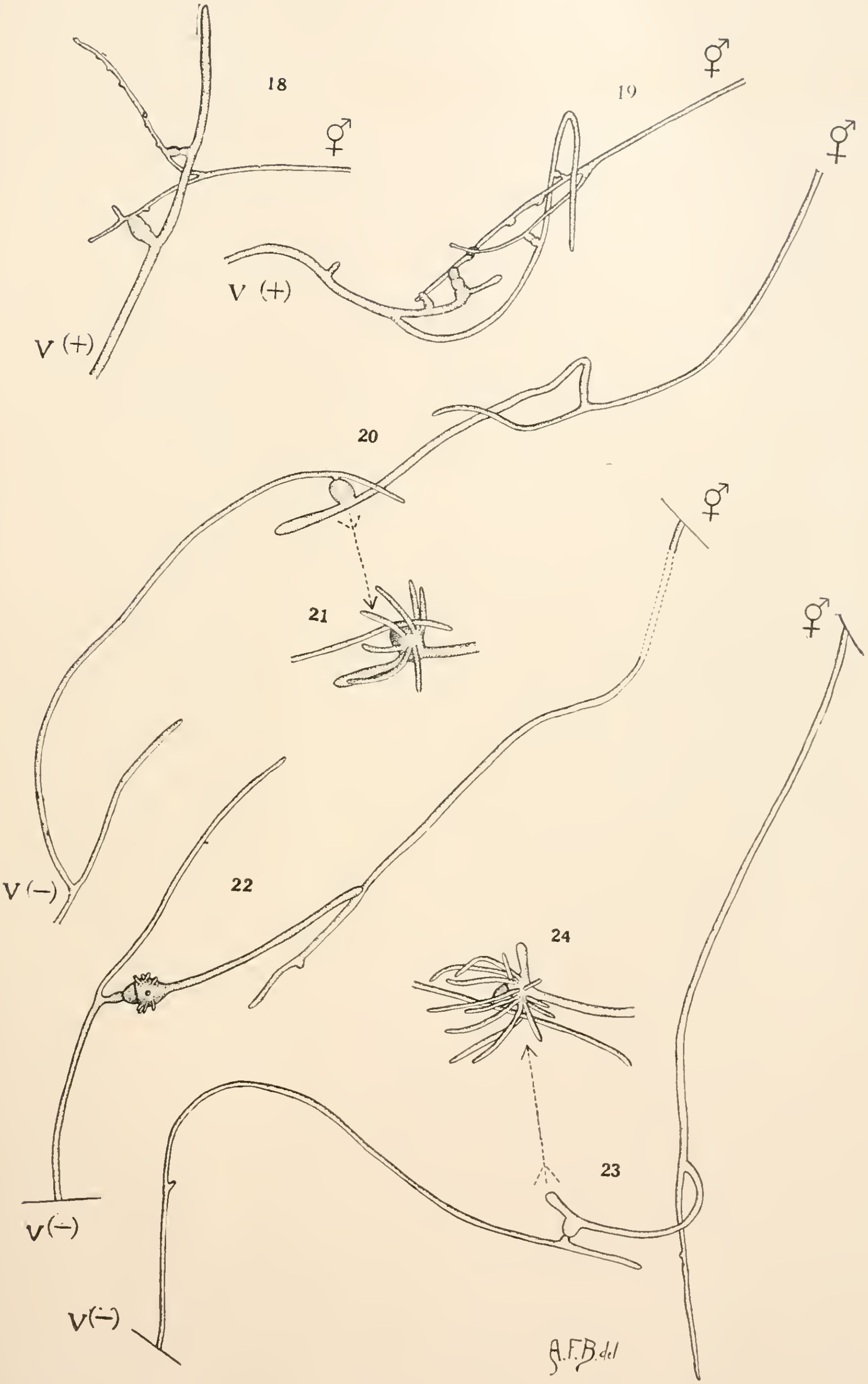
PLATE II.

FIGS. 18 AND 19. "Imperfect hybridizations" between *Mucor V.* (+) and *A. spinosa* (☿) drawn from living material.

FIGS. 20–24. "Imperfect hybridizations" between *Mucor V.* (−) and *A. spinosa* (☿). Fig. 20 drawn at 7.30 A.M. Fig. 21, the same at 3.30 P.M., Sept. 12. Fig. 23 drawn P.M., Sept. 12. Fig. 24, the same, A.M., Sept. 13.


18
⚥
V (+)
19
⚥
V (+)
⚥
20
21
⚥
⚥
V (–)
22
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V (–)
V (–)
A.F.B. del

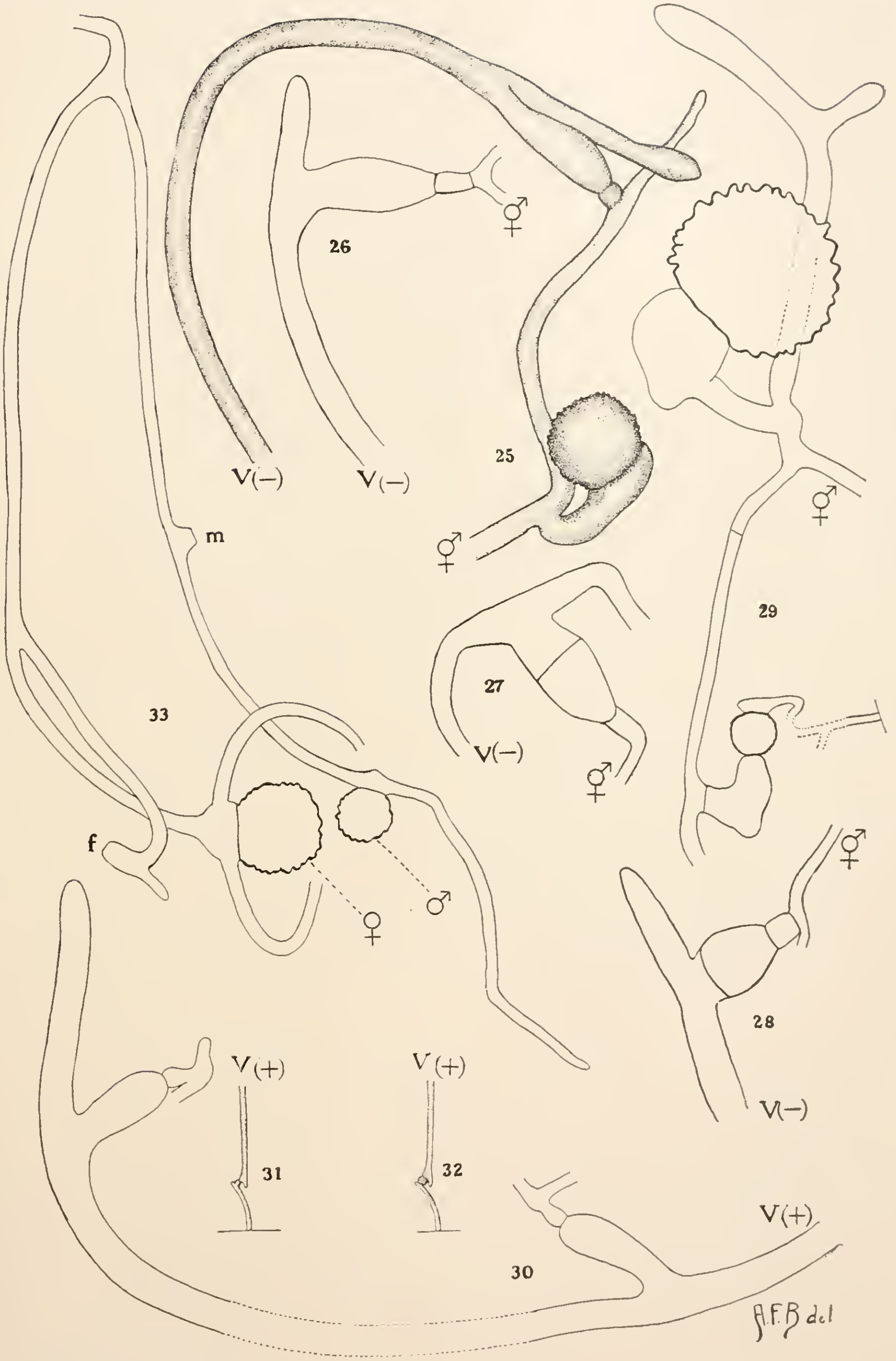
Plate III.

Figs. 25–28. "Imperfect hybridizations" between *Mucor V.* (−) and *Zygorhynchus heterogamus* (☿). Fig. 25 from living material. Figs. 26–28, gamete formation.

Fig. 29. "Imperfect hybridization" between *Zygorhynchus Vuillemini* var. *agamus* and *Zygorhynchus heterogamus* showing formation of azygospore from gamete of former species.

Figs. 30–32. "Imperfect hybridizations" between *Mucor V.* (+) and *Zygorhynchus Vuillemini* var. *agamus*. Fig. 30, progamete formation. Fig. 31 drawn at 9.55 P.M., Dec. 20. Fig. 32 drawn at 10.45 A.M., Dec. 21.

Fig. 33. Apposed azygospores of *Absidia spinosa* developed from male and female gametes. *m* and *f* apparently represent respectively male and female progametes which have been pulled apart in mounting.


26
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V(−)
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V(−)
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A.F.B del

ALBERT FRANCIS BLAKESLEE.

REVERSIBLE ACTIVATION AND INCOMPLETE MEMBRANE FORMATION OF THE UNFERTILIZED EGGS OF THE SEA URCHIN.[1]

JACQUES LOEB.

1. The writer showed in 1913 that the artificial activation of the egg can be reversed under certain conditions.[2] When the egg of *Arbacia* is treated with a base in the proper concentration and for the proper length of time the effect is somewhat similar to that which follows the treatment of the egg with a fatty acid. The egg is induced to develop, it may segment (as a rule abnormally) once or twice, and if nothing else happens it will perish rather rapidly (unless the developmental processes are prevented). If the eggs after the treatment with the acid or with the base receive a treatment with a hypertonic solution of the proper concentration and for the proper time they may develop into larvæ.

The writer found that if the eggs are treated with alkali in the proper way to induce development and if they are immediately afterwards put for several hours into a solution which prevents their development (sea-water with chloral hydrate or NaCN) the eggs when taken out behave as if nothing had been done to them. They neither segment nor do they disintegrate and they can again be induced to develop by fertilization (or probably with the methods of artificial parthenogenesis though the writer has not yet tried this). The activating effect of the alkali is therefore reversible.

This reversibility is, however, only possible if the eggs have not been exposed to the alkali too long. After too long an exposure the effect of the alkali is no longer reversible by a temporary suppression of the developmental processes. A second condition for the reversibility is that the eggs are immediately transferred from the alkaline solution into the chloral hydrate or NaCN solution. If they are first transferred into normal sea-water and then after fifteen or thirty minutes later are transferred to the cyanide solution nothing may happen to them as long as they remain in the cyanide solution (unless the HCN evaporates) but they will disintegrate when put back into normal sea-water. From this we must conclude that in the sea-water the processes started in the alkaline solution will continue and the egg behaves as if it had been overexposed to the hyperalkaline solution.

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[1] From the Rockefeller Institute for Medical Research, New York.

[2] Loeb, *Science*, N. S., XXXVIII., 749, 1913; *Arch. f. Entwcklngsmech.*, XXXVIII., 277, 1914.

Somewhat similar experiments were made with the eggs of *Arbacia* which had been treated with butyric acid. When such eggs were put over night into the cyanide-sea-water immediately after the artificial membrane formation they did not as a rule disintegrate when put back into normal sea-water but could be fertilized afterwards. The writer is, however, not certain that the phenomenon of reversibility is as constant here as in the case of the alkali treatment.

The writer pointed out that the explanation for this phenomenon could probably be found if we compare the behavior of the eggs of *Arbacia* with that of eggs of *purpuratus* under similar conditions. If we cause the production of a butyric acid membrane in the egg of *purpuratus* the activation of the egg is usually irreversible. In the egg of *purpuratus* the membrane which is formed under the butyric acid treatment is very tough and is separated by a wide area from the protoplasm, while in the egg of *Arbacia* the membrane consists often only of a fine gelatinous film which lies tightly around the egg. The writer concluded from this that there may be a quantitative if not a qualitative difference in the amount of change produced by the butyric acid treatment in the cortical layer of the two kinds of eggs; in the egg of *Arbacia* where this change is quantitatively smaller the activation is reversible, while in the egg of *purpuratus* where the change is quantitatively larger (and possibly also qualitatively different) it is irreversible (Loeb, "Artificial Parthenogenesis and Fertilization," Chicago, 1913, p. 286).

2. This idea is further supported by the curious phenomena of reversibility in the eggs of *Strongylocentrotus purpuratus*. When one tries to induce artificial parthenogenesis in the eggs

of *purpuratus* by a treatment with a hypertonic solution (without artificial membrane formation) it is found that this is possible only with the eggs of certain females. Such eggs develop into plutei. In the eggs of other females a small percentage of eggs will begin to segment and they may go to the 2, 4, 8, or 16-cell stage or still a little further but then stop developing. Such eggs will go into the resting stage again and are normal to all external appearances. If fertilized with sperm a number of hours or a day later the individual blastomeres will each form a special fertilization membrane and develop into small but apparently perfect larvæ.[1] This experiment shows that the activating effect which the hypertonic solution had was reversed, since all these eggs were at first in the state of active development.[2]

3. The writer wishes to report in this paper the fact that in the egg of *purpuratus* under definite conditions a membrane formation can be produced by butyric acid which leads neither to any development if followed by the usual treatment with a hypertonic solution nor to a rapid disintegration when not followed by any "corrective" treatment. Since in all previous observations on the effects of membrane formation by butyric acid the reverse was found it seemed of theoretical importance to study this exception.

The main condition for the experiment is that the eggs are put after the treatment with butyric acid into a $m/2$ solution of $NaCl + KCl + CaCl_2$ instead of into sea-water; the various salts are always used in the proportion in which they are contained in sea-water. The eggs were first washed three times in a mixture of $m/2$ $NaCl + KCl + CaCl_2$ and were then put for from one and a half to two and a half minutes into a mixture of 50 c.c. $m/2$ $NaCl + KCl + CaCl_2$ + 1 c.c. $N/10$ butyric acid; from here they were transferred into a neutral or a slightly alkaline solution of $m/2$ $NaCl + KCl + CaCl_2$. In this solution all the eggs form a membrane which is very thin and which is at first at a great distance from the egg. Figs. 1 and 2 give an idea of the first appearance of this membrane; the membrane in Fig. 1 was formed in a neutral, that of Fig. 2 in a faintly alkaline solution. Later on, the membrane collapses and is found lying rather close to the egg. Such eggs can be readily fertilized with sperm in spite of the existence of the membrane. The latter is either naturally permeable for sperm or it is easily torn and thus allows the sperm to reach the egg or it is not entirely continuous around the eggs.

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[1] Loeb, *Arch. f. Entwcklngsmech.*, XXIII., 479, 1907; "Artificial Parthenogenesis and Fertilization," Chicago, 1913, p. 237.

[2] Only the activating effect of the hypertonic solution is reversible, the second, corrective effect, which the hypertonic solution imparts to the egg was not reversed since these blastomeres develop also if only the artificial membrane formation is induced in them by butyric acid, while without the previous treatment with a hypertonic solution such eggs would soon perish. Loeb, *Jour. Exper. Zool.*, XV., 201, 1913; "Artificial Parthenogenesis and Fertilization," p. 238.

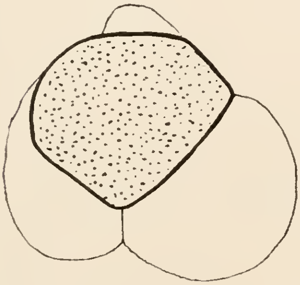

FIG. 1.

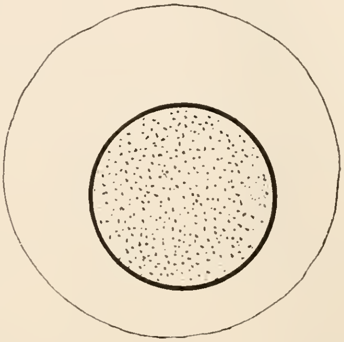

FIG. 2.

Eggs were washed three times in a mixture of $m/2$ NaCl + $CaCl_2$ + KCl and then put for from one and a half to two and a half minutes into 50 c.c. $m/2$ NaCl + KCl + $CaCl_2$ + 1.0 c.c. $N/10$ butyric acid. From this solution they were transferred:

(*a*) Into normal sea-water.[1]

(*b*) Into a neutral mixture of $m/2$ NaCl + KCl + $CaCl_2$.

Lot *a* formed normal fertilization membranes and disintegrated in a few hours. The eggs of lot *b* formed the fine fertilization membrane which was at first very distant from the egg. Some of these eggs were transferred into normal sea-water after one hour. These eggs did not form a better membrane but perished in the same way as the eggs of lot *a*.

A second portion of lot *b* was transferred into normal sea-water after having been for seven hours in the mixture of $m/2$

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[1] Such eggs have at first a tendency to stick to the glass when transferred to sea-water. It is necessary to keep them in slight agitation for some time until they have lost their tendency to stick.

$NaCl + KCl + CaCl_2$. These eggs did not disintegrate during the following night although they did not appear quite normal the next day. The rest of the eggs remained in the $m/2$ $NaCl + KCl + CaCl_2$ solution for twenty-four hours. When these eggs were put into sea-water they did not disintegrate. To some of these eggs sperm was added the next morning and about 20 per cent. developed.

This experiment indicates that the nature of the membrane and the cortical changes which activate the egg of *purpuratus* are to a large extent determined by the solution into which the eggs are put after the butyric acid treatment. If the eggs are put directly into sea-water after the butyric acid treatment they form the typical membrane and rapidly perish if nothing else happens to them. The same may be the case if they are transferred from the butyric acid solution into a neutral solution of $m/2$ $NaCl + KCl + CaCl_2$ for a short time (less than one hour) and if they are afterwards transferred into normal sea-water. But if they remain in the $NaCl + KCl + CaCl_2$ solution for a number of hours they will as a rule not disintegrate if put back into sea-water.

A second experiment may be quoted. Eggs (that had been washed three times in a neutral $m/2$ $NaCl + KCl + CaCl_2$ solution) were put for one and a half to two and a half minutes at a temperature of about 14° into 50 c.c. of the same solution + 1 c.c. $N/10$ butyric acid. From here the majority of the eggs were transferred into a neutral solution of $m/2$ $NaCl + KCl$ $CaCl_2$ and the rest into sea-water. The latter formed normal fertilization membranes and disintegrated the same day. Those transferred into the neutral $NaCl + KCl + CaCl_2$ solution had all formed membranes which were at first separated from the egg by a great distance. They were kept in this solution over night and the next morning they were transferred into normal sea-water. All looked normal except that the membrane now formed a fine veil lying closely to the egg. Some of these eggs were fertilized with sperm. They all segmented and developed into swimming larvæ. The others were kept in sea-water without sperm to find out whether they would now disintegrate or whether the effect of the butyric acid treatment had worn off. This was the case. The eggs no longer disintegrated in sea-water. A fraction of these eggs was fertilized later and segmented.

When the eggs are transferred into normal sea-water immediately after the butyric acid treatment they perish rapidly if no second treatment is given to them. If they are treated with hypertonic sea-water (50 c.c. sea-water + 8 c.c. 2 1/2 *m* NaCl or NaCl + KCl + $CaCl_2$) for from thirty to sixty minutes they will develop into larvæ. If they are treated longer they will begin to develop but form very abnormal larvæ.

If the eggs, however, after the butyric acid treatment are not put into sea-water but into a mixture of *m*/2 NaCl + KCl + $CaCl_2$ in which they form the thin membrane and if they are kept here until they no longer disintegrate in normal sea-water, they cannot be induced to develop when treated for from thirty to sixty minutes with a hypertonic solution. Such eggs have, therefore, in spite of their membrane formation gone back into the condition of a resting egg.

4. It was obvious from these experiments that the constituents of the sea-water determine the nature of the membrane after butyric acid treatment. The writer expected to find that Ca was an absolute prerequisite for the membrane formation but this was not the case, although the presence of $CaCl_2$ improves the character of the membrane. Eggs that had been taken out of a *m*/2 NaCl solution were treated with 50 c.c. *m*/2 NaCl + 1 c.c. *N*/10 butyric acid for one and a half to two and a half minutes. They were then transferred into the following three sets of solutions:

(1). NaCl + KCl,
(2). NaCl + $CaCl_2$,
(3). NaCl + KCl + $CaCl_2$,

which were made in duplicates, the one set remaining neutral, the second set faintly alkaline (0.5 c.c. *N*/100 NaOH were added to 100 c.c. of the solution). The result was not very different in the neutral and alkaline solutions. Many but not all the eggs put into NaCl + KCl formed very thin membranes often only in part of the egg. The membrane formation does therefore not require the addition of $CaCl_2$, but the eggs transferred into NaCl + $CaCl_2$ or into NaCl + KCl + $CaCl_2$ had formed a better and apparently stronger membrane than those put into NaCl + KCl.

In another experiment it was found that in a mixture of NaCl + $MgCl_2$ in the proportions in which these two salts occur in the sea-water a membrane formation was also possible, but the membrane had the same fine and thin character. In all these cases the eggs could be fertilized with sperm in spite of the membranes. The writer is under the impression that no second membrane was formed upon the addition of sperm and only the hyaline membrane developed later. That the eggs after this inefficient membrane formation were not in the same condition as eggs which are put into normal sea-water after artificial membrane formation was shown by the fact that when put into a hypertonic solution for from thirty to sixty minutes not a single egg could be induced to segment.

The fact that the membrane always collapsed after some time seems to indicate that it soon became permeable for the colloids liquefied at the surface of the egg in the process of membrane formation.

5. The explanation of the phenomenon of ineffective membrane formation will probably be the same as that for the cases of reversible activation of the egg. It is an established fact that the rate of oxidations in the egg of *purpuratus* is raised about 600 per cent. by the act of fertilization. This rise is entirely due to the alteration of the surface layer of the egg which results in the membrane formation since artificial membrane formation alone has the same effect on oxidation. Even if the egg is killed in the process of membrane formation, *e. g.*, if we treat the unfertilized egg with saponin and leave it in this solution, the rate of oxidation is raised to the same amount.[1] The amount to which the artificial membrane formation raises the rate of oxidations in a sea-urchin egg seems to be a constant for each species and not to depend upon the nature of the agencies employed for this purpose.

When we treat the unfertilized eggs of *purpuratus* with a hypertonic solution (without inducing a membrane formation) the

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[1] Loeb and Wasteneys, *Jour. Biol. Chem.*, XIV., 479, 1913.

eggs of some females will develop into larvæ, while the eggs of others will not. The writer assumed that the hypertonic solution if applied to the unfertilized egg has two effects, one consisting in the alteration of the surface layer (comparable to the effect of butyric acid) and secondly the corrective effect. Wasteneys and the writer[1] published a series of measurements on the influence of a hypertonic solution on the rate of oxidations in unfertilized eggs of *purpuratus*, and found that the effect varied enormously with the eggs of various females. The eggs were in the hypertonic solution for one hour and the rate of increase in oxidation varied for the eggs of various females between 40 and 400 per cent. As we stated above, the eggs of only a limited number of *purpuratus* can be induced to develop into larvæ by a mere treatment with a hypertonic solution and it is probable that the eggs in which the oxidations were raised efficiently were the ones that could be induced to develop into larvæ in this way, while those in which the rate of oxidations was not raised to the same height did not segment. We also noticed that in the latter type of eggs the rate of oxidations diminishes after some time. It is possible that the reversion of development in such cases is due to a decline in the rate of oxidations below the height required for development.

The same possibility holds for the lack of development of the eggs after artificial membrane formation through butyric acid when they are afterwards put into a solution of $NaCl + KCl + CaCl_2$ (instead of into sea-water). The fact that no correct membrane is formed and that the eggs neither develop nor disintegrate, and behave towards a treatment with the hypertonic solution like eggs without a membrane, arouses the suspicion that in this case the butyric acid treatment did not lead to the proper increase in the rate of oxidations. It is intended to investigate this possibility next summer, since it may also furnish the explanation of the phenomena of reversibility in development.

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[1] Loeb and Wasteneys, *Jour. Biol. Chem.*, XIV., 474, 1913.

REVERSIBILITY OF THE REACTIONS OF PLANARIA DOROTOCEPHALA TO A CURRENT OF WATER.

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I. INTRODUCTION.

A reaction of planarians to currents of water has been described by Pearl ('03) in his careful study of the general features of the behavior of planarians. He has described the reaction and his method of obtaining it as follows: "In the course of the experiments to localize chemical stimuli by the capillary tube method, it was discovered that by means of a tube with a relatively large opening (from .25 to .50 mm. in diameter) and letting the ordinary tap-water in which the animals were flow out of it by its own weight, a current of just the right intensity to cause a positive reaction could be produced. The animals would turn very sharply toward the source of such a current, the reaction being evidently the same as that to other weak stimuli (chemical and mechanical). This reaction is localized in the same way as the usual positive reaction. It is given only when the current is directed against the head or anterior part of the body" (p. 698). He states that earlier in his work a large number of experiments were performed with various devices to determine whether these animals would show such a reaction, but without success. Streams of water from a pipette and similar devices caused only a stopping, longitudinal contraction, and gripping of the bottom without any turning either toward or away from the source of the stimulus.

That the rheotropic reactions of planarians were found so difficult to demonstrate, it seems probable, must have been due to the experimental methods employed. Using the methods described below, it has been found that rheotropic reactions of these animals can be demonstrated very easily, not only in a "current of just the right intensity" but in currents of a large range of intensities. Under the conditions of these methods a worm is entirely surrounded by the flowing water on all surfaces except the ventral surface which is attached to the substratum, and the conditions of stimulation are more typically rheotropic than when the stream of water is directed as a small jet against a localized part of the body. A negative reaction, *i. e.*, a turning away from the side stimulated, was not described by Pearl but Dr. C. M. Child, who suggested this study, has observed a negative reaction as well as a positive reaction in currents of water in his laboratory stocks of worms used in studies in regeneration. It has been found that these reactions are reversible experimentally. The study of their reversibility which is reported in the present paper was preliminary to a more detailed study of the rheotropism of these animals which is in progress at the present time.

The work reported in the present paper was done some time ago in the zoölogical laboratory of the University of Chicago. My acknowledgments are due Dr. C. M. Child and Dr. V. E. Shelford for helpful suggestions.

II. MATERIAL AND METHODS.

Planaria dorotocephala has been used exclusively for this study. Specimens were collected from a spring-fed marsh at the margin of the Fox River near Chicago, Illinois. These animals are very easy to keep in the laboratory without special care. Since this study was in the nature of a testing of the reaction possibilities of the worms toward currents of water, rather than an effort to determine the normal habits, no efforts were made to duplicate the normal conditions of existence in nature. Specimens were kept in large numbers in glass and galvanized iron containers which were emptied of water, rinsed and filled with fresh tap-water from time to time without removing the worms which cling to the surface of the vessel. They were fed two or three times a week with fresh beef cut into small bits and left in the dish for several hours or during the night. These worms collect on fresh meat and secure blood and juices but it is very improbable that they are able to make use of the solid portions.

Rheotropic reactions were tested in a "circular current" as follows: A considerable number of specimens, sometimes several hundred, were placed in a circular shaped vessel such as a glass battery jar or a kitchen pan and the water was stirred vigorously around the dish several times with a stirring rod. Vigorous stirring usually dislodged the most of the specimens which were then swept into a bunch at the center of the current. If they were not dislodged by the stirring, they were loosened by means of more vigorous streams of water from a large bulbed pipette. The movements of the animals on the bottom of the vessel only were studied since those on the sides were in a different relation to gravity which introduced a geotropic factor into the reaction. Rheotropism has been observed, however, in worms gliding on vertical surfaces.

It was found convenient to have all the specimens, or the larger part of them, enter the experiment at the center of the "circular current" since they were then all placed under similar conditions. In whatever direction any worm started out from the center, it would receive the current against the same side of the body as all the other worms starting from the center. If the current were stirred in the clockwise direction, for example, all the worms starting from the center of the dish would receive the current against the left side of the body. If they gave a positive reaction, they turned toward the left side; that is, toward the side stimulated, or up-stream, and if they gave a negative reaction, they turned toward the right side; that is, away from the side stimulated, or down-stream. When a large number of worms were gliding on the bottom of the dish, if their reactions were uniform, a very striking figure was produced.

This is illustrated in Figs. 1 and 2. Fig. 1 is a photograph of a lot of worms in a dish pan, reacting positively to a current stirred in the clockwise direction. The conspicuous spiral form of the figure is characteristic of the positive reaction. Fig. 2 is a photograph showing the characteristic negative reaction near the center of the pan.

These peculiar and characteristic spiral figures in a "circular current" called for a more careful examination of the physical conditions in the experiment, which revealed the fact that the worms were subjected to a system of spiral currents instead of a circular current. If a drop of a water suspension of carmine is placed on the bottom of a dish in which the water has been stirred in this way, the carmine in the lower layers of water close to the bottom will be seen to stream inward along a spiral course toward the center. Fig. 3 is a photograph of a dish pan in which the water was stirred in the clockwise direction and then drops of carmine were placed on the bottom at eleven points around its circumference. The carmine was dragged along by the currents and left streaks on the bottom of the pan which show as spiral lines in the photograph. The worms were subjected to this spiral system of currents. Carmine in the upper layers of water was swept about the dish in a fairly circular direction, and the water which flowed along the spiral lines on the bottom toward the center, rose, as it neared the center, and spread outward above.

A comparison of Fig. 3 with Fig. 1 shows that the spiral lines of the currents and the spiral lines of the positive reaction are alike. This explains, therefore, the spiral character of the reaction. The worms orient themselves to a spiral current. A comparison of Fig. 2 with Fig. 3 shows that in the usual negative reaction the direction taken is away from the side stimulated, but rather diagonally than directly down-stream. In some cases, however, when worms were distributed over the bottom and were not dislodged by the stirring, they were observed to turn inward and follow along the lines of the spiral current in as precise a negative orientation as was often characteristic of the positive reaction.

A mixture of definitely positive and definitely negative reactions at the same time, that is, an intermingling of the two systems of spirals, was not common. The reactions varied, however, in uniformity at different times; that is, in the precision of orientation, and in some cases the worms scattered from the center without forming any very definite spiral in either direction. Such "reactions" were designated "indefinite."

III. Difference of Reaction to Currents of Different Velocities.

In studying the reversal of rheotropic reactions induced by changing the chemical and thermal conditions, it was found that it is important to consider the velocity of the current. A change in the velocity of the current, or, in other words, in the strength of the rheotropic stimulus, may itself cause reversal of the sign of the reaction.

Since a current in a small vessel diminishes rapidly in velocity after the stirring is discontinued, the different worms in a single trial are subject to different velocities of current, the first worms to leave the center entering the strongest current and later worms finding successively weaker current. It was frequently observed that the positive reaction was given by the earlier worms; that is, those in the strongest current, and the negative reaction by the later worms; that is, those in the weaker currrent. The proportion of individuals giving each reaction varied in different observations while in some cases no negative reaction was given and in other cases no positive reaction was given.

A positive reaction in stronger current and a negative reaction in weaker current can be seen at the same time in a broad-bottomed pan where the negative spiral may begin about the center before the last individuals belonging to the positive spiral have reached the sides. This is possible because the current is swifter about the outside where the positive reaction is still being given than about the center where the negative reaction is beginning. Fig. 2 shows such a combination of positive spiral about the outside and negative spiral about the center. In the case which is photographed there is considerable irregularity in the positive spiral. In many cases the positive spiral about the outside was as regular as that in Fig. 1.

Table I. shows the total number of trials in which the different types of reaction were given during all the experiments.

TABLE I.

SUMMARY OF ALL REACTIONS OBSERVED (EXCEPT THOSE GIVEN UPON INCREASING THE VELOCITY OF THE CURRENT, FOR WHICH SEE TABLE II).

The sign + indicates a positive spiral, −, a negative spiral and ?, no dominant spiral of either sign. The sign + − indicates positive reaction in stronger current and negative reaction in weaker current; (+) − indicates only a few worms in the positive spiral, and + (−) only a few worms in the negative spiral.

Reactions.	Total Number of Trials of Worms in Normal Conditions Preceding Experiments, and in Normal "Controls" During Experiments.	Total Number of Trials During Experiments, in all Conditions, Excepting the "Controls."	Totals.
+	324	119	443
−	31	130	161
(+) −	45	48	93
+ −	46 } 113	36 } 104	82 } 217
+ (−)	22	20	42
?	32	29	61
Totals.....	500	382	882

In the observations described above, the same individuals were not observed to give both reactions because under the conditions of the tests the same individuals are not subjected to both extremes of current velocity. The earlier worms receive the stronger current and the later worms receive weaker current. It was readily shown, however, that the same individuals which responded negatively in a weaker current would respond positively in a stronger current. Upon second stirring of the water over a negative spiral without dislodging the worms, it was generally found that the worms composing the negative spiral reversed their direction of movement from negative to positive, so that the negative spiral became converted into a positive spiral composed of the same individuals. Out of 100 cases in which second stirring of the water over a negative spiral was tried, 80 cases showed reversal to positive. In some cases all the individuals gave this reversal while in other cases few individuals, that is, only those forming the outside of the spiral gave the reversal. In some cases reversal was readily induced by currents of moderate velocity while in other cases reversal was given only in very strong currents.

TABLE II.

REVERSAL OF REACTION INDUCED BY INCREASING THE VELOCITY OF THE CURRENT. SUMMARY OF ALL REACTIONS IN EXPERIMENTS IN WHICH THE VELOCITY OF THE CURRENT WAS INCREASED WHILE A REACTION WAS BEING GIVEN.

Velocity Increased During a Negative Reaction.

Complete Reaction Before Increase in Velocity.	Reactions After Increase in Velocity.				
	Reversal to +.	More Precise —.	No Change —.	Worms Dislodged.	Reaction Became ?.
—	21	5	5	1	1
(+) −	18	1	2		
+	28		3	2	
+ (−)	13				
Totals....	80	6	10	3	1

Velocity Increased During a Positive Reaction.

Complete Reaction Before Increase in Velocity.	Reactions After Increase in Velocity.	
	More Precise +.	No Change +.
+	8	4
? +	1	

Velocity Increased During an Indefinite Reaction.

Complete Reaction Before Increase in Velocity.	Reactions After Increase in Velocity.
	Reaction Became +.
?	4
(+) ?	4
+ ?	15
+ (?)	1

No evidence has been obtained that a stronger rheotropic stimulus will cause a negative reaction while a weaker stimulus causes a positive reaction. No observations showed a sequence of reactions during the diminution of the velocity of the current; of negative in stronger current and positive in weaker current (*cf.* Table I.). And second stirring of the current while the worms were distributed over the bottom was never observed to reverse the reaction from positive to negative. If the worms were giving the positive reaction in weaker current they continued to give the same reaction in stronger current, and usually with greater precision of orientation (Table II.). When the reaction was "indefinite" in weaker current, the stronger current usually called forth the positive reaction.

IV. Reversal of Reaction Induced by Chemical Changes.

Reversal of reaction from positive to negative can usually be induced by pouring off the water from the worms and replacing it with fresh tap-water. Thus in 23 out of 24 observations this treatment resulted in reversal to the negative reaction. Two factors may be responsible for this reversal, change in temperature and change in the chemical composition of the water. Each of these factors was investigated by modifying the condition in question while preserving other conditions unmodified. The velocity of the current could not be preserved unmodified with the methods employed, but this factor was controlled indirectly.

The effect of changing the chemical character of the water was tested as follows: A lot of worms were tested in the water in which they were living at the time. Then an equal volume of fresh tap-water, or modified water, was brought to the same temperature as the aquarium water, and the worms were tested alternately in these two kinds of water.

A number of experiments were performed in which aquarium water was replaced by fresh tap-water of the same temperature. Since the rheotropic reaction in many cases may be positive in stronger current and negative in weaker current, it must be made certain that the reversal of reaction after a chemical change in the environment is a reversal of reaction in the same velocity of current. Although it seemed fairly certain to the operator, during the earlier experiments, that there was such a reversal in the same velocities of current, the later experiments were performed with a "control" to make them more conclusive. In these experiments two dishes of worms living under the same conditions, frequently in the same aquarium tank, were tested side by side. One lot of worms was tested in waters of different composition while the other lot was kept as the "control" and was tested always in the original aquarium water. The experimental and control dishes were placed side by side, and the control worms were tested at each trial simultaneously with the experimental worms so that the conditions as regards velocity of current were practically the same in the two dishes. Progressive diminutions in velocity were practically the same in the two dishes. Differences of reaction given by the worms in the two dishes at the same time of observation must then be traced to other factors than the velocity of the current.

The following experiment will illustrate the method of the controlled experiments and the characteristic reversal of reaction induced by change from aquarium water to fresh water. Many hundreds of worms of miscellaneous sizes were tested in two large galvanized iron tanks. A current was produced by shaking the tanks in the hands, which swept the worms to the center. In the experimental tank aquarium water was used in trials 1, 3 and 5; and this was replaced by fresh water in the alternate

Table III.

Experiment Showing Reversal of Reaction from Positive to Negative Induced by Change from Aquarium to Fresh Water at the Same Temperature.

Trial.	Experiment.		Control.
	Water.	Reactions.	Reactions.
1	Aquarium	+ good general response	+ in strong current — in weak current
		+ on second stirring, even in weak current	+ on second stirring, universal.
2	Fresh	— even in fairly strong current.	+ even in very reduced current.
		— on second stirring, even in fairly strong current, a few + in strongest current	
3	Aquarium	— slight; worms slow in distributing on the bottom.	+ even in very weak current.
		+ on second stirring, even in only moderately strong current.	
4	Fresh	— even in fairly strong current.	+ even in very weak current.
		— on second stirring, no positive reaction.	
5	Aquarium	movement very slow, current reduced before any reaction was given.	+ even in very weak current.
		+ on second stirring only moderately strong current.	

trials 2 and 4. In the control tank the worms remained in the aquarium water in all the trials. The temperature was 21° C. The reactions in each trial are described in Table III.

From the table it can be seen that placing the worms in fresh water reversed the reaction from positive to negative while returning them to their former aquarium water brought back the former positive reaction. These reactions were reversed back and forth by changing the water. The reversal of reaction cannot be attributed to a difference in the velocity of the current. The negative reactions in the fresh water were given in fairly strong current while the positive reactions in aquarium water were given in very weak as well as stronger current; and in the control, in which the velocity of the current was approximately the same at each moment as that in the experiment, a positive reaction was given at the same time that a negative reaction was observed in the experiment. In the two tests in this experiment with fresh water (trials 2 and 4), therefore, a change to fresh water induced a reversal of reaction from positive to negative while a return to the normal environment brought back the positive reaction. In 40 such tests in experiments at different times, a reversal from positive to negative was given in 21 tests and a reversal from negative to positive in 5 tests. In 7 tests a positive reaction remained unchanged while in 5 tests a negative reaction remained unchanged. In 2 tests the reaction was "indefinite."

Fresh water differs from aquarium water in oxygen content and in the absence of the metabolic products of the worms and the decomposition products that accumulate in the aquarium. The aquarium water in experiments at different times varied greatly in composition, while the fresh drawn tap-water probably varied little. It would seem that fresh water would make a more suitable environment than stale aquarium water where many worms have lived together without green vegetation. In the experiment described above the aquarium water was quite foul from the decomposition products of juices of meat that had stood in the aquarium for nearly 24 hours. When modified aquarium water was used instead of fresh water, reversal of the same sort was induced. In one experiment part of the aquarium water was boiled, and in the two tests made with this boiled aquarium water reversal of reaction from positive to negative was obtained. In another experiment cane sugar was dissolved in part of the aquarium water to make a 1 per cent. solution which induced reversal from positive to negative in the 6 tests made. Similarly a well-mixed solution of juices of raw beef in aquarium water induced the same reversal in the 6 tests made. So far as these experiments indicate, therefore, the reversal of the rheotropic reaction in the new environment seems to be due more to the strangeness of the new conditions than to their fitness or unfitness. In a total of 54 tests with modified aquarium water and fresh water, reversal of reaction from positive to negative was induced in 35 tests, while in 5 tests the reaction was already negative from other causes. These figures may not necessarily represent the proportion of individual worm reactions which can be reversed in this way, since the conditions in different experiments were not entirely identical, but it may be repeated that in each of these tests a large number of worms were tested, in some cases many hundreds of individuals.

V. Reversal of Reaction Induced by Changes of Temperature.

The effect of changes of temperature upon the rheotropism was tested by dividing the water in which worms were living into two portions, preserving one portion at the temperature at which the worms were found at the beginning of the experiment, and cooling or warming the other portion to the desired temperature. The worms were then tested alternately in these two portions of water. It was found that the rheotropism can be reversed from positive to negative by lowering the temperature in the same manner as by changing the composition of the water.

In 34 individual tests, lowering the temperature by 4°–10° C. was found to cause a reversal in 17 tests from positive to negative, and reversal from negative to positive in 2 tests. In 4 tests this change in temperature made an indefinite reaction become positive. In the remaining 11 tests, lowering the temperature produced no observed change in reaction. This remained as before the temperature change; that is, as follows: positive in 7 tests, negative in 3 tests, positive in stronger current and negative in weaker current in one test.

The characteristic rheotropic effect, therefore, of lowering the temperature below that to which the worms are accustomed is a reversal of reaction from positive to negative. Return to the normal temperature brings back the positive reaction so that the reactions can be reversed back and forth by alternately lowering the temperature and raising it again to the normal. The reversal from negative to positive on raising the temperature seems to be due to the return to the temperature to which the the worms are accustomed, rather than to the fact that the temperature is raised, since in 13 tests of raising the temperature above the normal by 4°–10° C. reversal from negative to positive was obtained in only one case. In 4 tests the reversal was in the opposite direction; that is, from positive to negative. In 5 tests the negative reaction remained unchanged and in 2 tests the positive reaction remained unchanged, while in one test the reaction became "indefinite."

VI. Variations in Rheotropic Reactions.

The general features of the rheotropic reactions of planarians and their reversibility described above were observed in mass tests of large numbers of individuals. These depend upon considerable uniformity in the behavior of the different individuals in the trial, much as do the behavior experiments with cultures of protozoa. The uniformity of the reactions of the different individuals in a single trial in the experiments with planarians was generally very striking, at least with respect to the sign of the reaction in the same velocity of current. The precision of the reactions of different individuals varied greatly in different trials, although the sign of the reaction was definite. When the reaction is definitely either positive or negative, the characteristic spiral figure is very striking, and when a spiral could not be seen, the reaction was called indefinite. Only a small number of trials, however, failed to show a general uniformity in the sign of the reaction in the same velocity of current (Table I.).

The different tests of reversal in the same experiment generally gave fairly consistent results although with some variations among the different tests. But in some experiments the lot of worms gave consistent results directly opposite to those generally obtained. Thus in the experiments of lowering the temperature, five of the seven tests in which the positive reaction remained unchanged were the successive tests in a single experiment. In this case the temperature at the beginning of the experiment, and the amount of the depression of the temperature, were the same as in other experiments in which reversal of reaction was produced in the characteristic manner. Such differences in behavior must be attributed to that complex of internal processes which has been characterized the "physiological state" of the organism.

VII. Reactions to Currents of Water in Nature.

That rheotropic reactions are normal to the life of planarians in nature is shown by field observations. The specimens studied in the laboratory were collected in a spring-fed marsh at the edge of the Fox River near Chicago, called "Station 10 Cary Spring" in Shelford's description of the animal communities of the Chicago Region (Shelford, '13, pages 52, 93 and 118). The marsh bottom is composed of black humus without stones under which the worms could collect in the manner that Pearl found characteristic of this species in the Huron River, but an abundance of leaves serves the same purpose. The water is very shallow and well shaded by marsh vegetation. There is a sluggish current fed by springs at the upper edge of the marsh, where water cress is found growing.

A small channel about five inches wide and fifteen feet long was discovered at the edge of the marsh through which a moderate current of water was flowing. A large number of worms were found in this stream, some moving on the bottom, generally up-stream, but most of them at rest on the under sides of leaves or in sheltered places on the bottom. Removing the shading vegetation for better observation stimulated many of the worms at rest to become active, and half an hour later 60 worms were counted going up-stream and 5 going down-stream and 200 were counted at rest on the under side of leaves. At that time the sun was shining somewhat from the side, and down-stream, but it was not far from vertical as it was 11:30 in the morning. At 12:20 only 4 worms could be found in sight, 3 going up-stream and one going down-stream. At 1:30, when it was cloudy, 40 worms were found going up-stream and 4 going down-stream. At 3:10 a piece of raw beef was placed in the upper part of the stream and fifteen minutes later 176 worms were counted going up-stream and 34 going down-stream. It has frequently been noted that worms in a moderate stream of water will respond in this way and collect upon a piece of fresh beef and this behavior has been put to practical advantage in collecting specimens. Worms removed from this stream and tested in a circular pan by the method employed in the laboratory showed both the positive and the negative reactions very clearly, the positive in stronger current and the negative in weaker current, as described above.

These observations show that planarians may react to currents of water in nature. Pearl considered it "very improbable that this reaction is of any importance in the normal activity of the animal." This opinion may have been due to the difficulty which he experienced in demonstrating the reaction experimentally which might lead one to think that unusual conditions are necessary for its production in nature; whereas it has been shown that the reaction is given with readiness under experimental conditions which are similar to those in a natural stream of water, and also that it can be observed in nature. It would seem that a reaction which is so characteristic of the animals in the laboratory would play a part in their daily life in nature, when they live in an environment which offers the appropriate stimuli for the reaction. These animals seem to belong characteristically to stream communities where these conditions are fulfilled.

VIII. Summary.

1. *Planaria dorotocephala* show both positive and negative reactions in a stream of water.

2. The sign of the reaction may differ, depending upon the velocity of the current. The positive reaction is then given in stronger current and the negative reaction in weaker current.

3. A negative reaction in weaker current can often be reversed to the positive by increasing the velocity of the current.

4. Reversal of reaction from positive to negative can be induced by changing the composition of the water; and from negative to positive by return to the former conditions.

5. Similar reversals of reaction can be induced by sudden changes in temperature.

6. Rheotropic reactions are given by *Planaria dorotocephala* in nature.

7. Rheotropic reactions are very characteristic of these animals in the laboratory, rather than unusual as has been supposed.

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EXPLANATION OF PLATES.

PLATE I.

FIG. 1. The positive spiral. Photograph of worms giving a positive reaction to a current of water stirred in the clockwise direction.

FIG. 2. The negative spiral. Photograph of worms giving a negative reaction to a current of water stirred in the clockwise direction. The worms near the center are reacting negatively while those about the outer part of the pan, in the stronger current, are reacting positively.

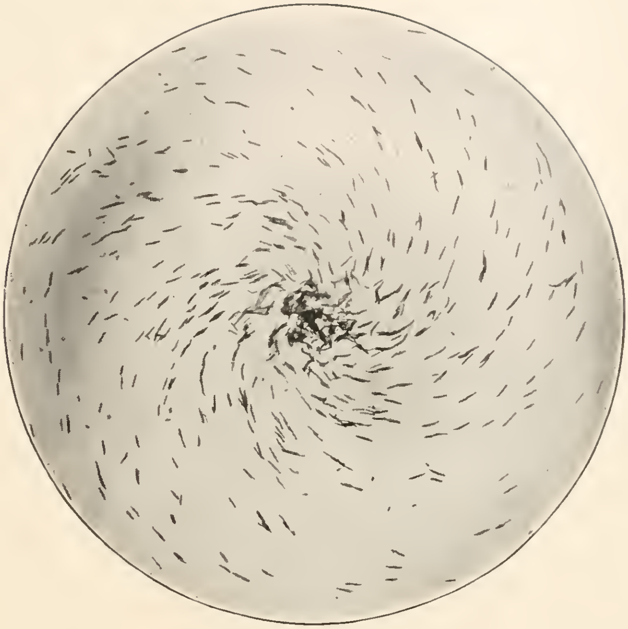

FIG. 1.

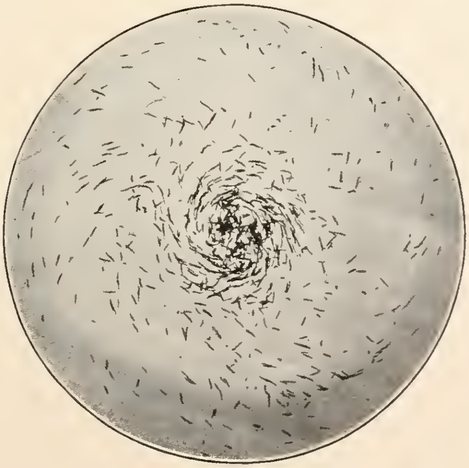

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FIG. 2

PLATE II.

FIG. 3. The system of spiral currents. Photograph of pan in which the water has been stirred in the clockwise direction and then drops of carmine have been placed on the bottom at various points about its circumference. The dark lines are streaks of carmine produced by the currents and showing the direction of the currents upon the bottom.

FIG. 4. A positive reaction showing individual variation in the precision of orientation.

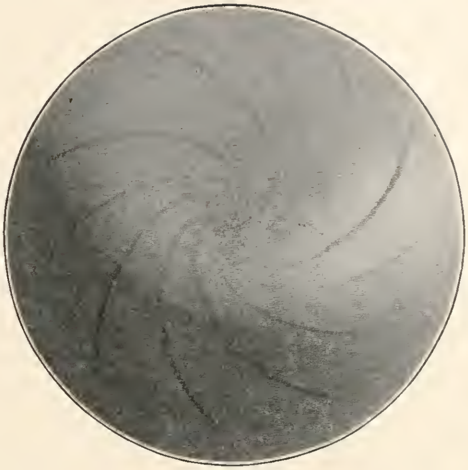

FIG. 3.

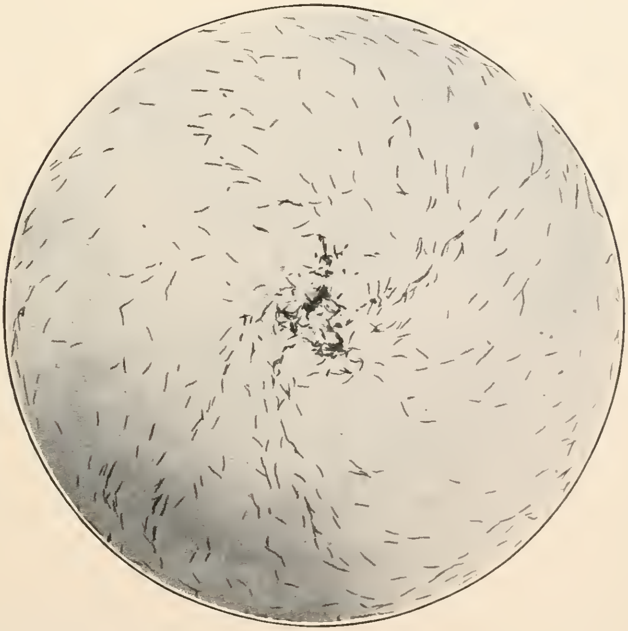

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FIG. 4.

A CASE OF HERMAPHRODITISM IN SPELERPES BISLINEATUS.

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Hermaphroditism among *Anura* seems to be comparatively common. Ecker and Wiedersheim's "Anatomie des Frosches," as revised by Gaupp, devotes several pages to the subject, dividing it into various types, the most common being that in which the glands are essentially male with female elements.

Miss King ('10) in her paper on anomalies in the genital organs of toads, says: "Evidently hermaphroditism occurs much less frequently among the *Urodela* than among the *Anura*, as only two cases have as yet been reported for this group of Amphibians. La Valette St. George ('95) has given a brief description of a case of hermaphroditism in *Triton tæniatus* and Knappe ('86) has noted the presence of a Bidder's Organ in a young salamander; neither investigator gives any details regarding the structure of the ovo-testes in these forms."

Knappe mentions *Triton* also in his investigations but seems to have found a Bidder's Organ only in a two-year-old *Salamandra maculata*. Concerning the frequency of occurrence of hermaphroditic salamanders, La Valette St. George says: "Für die Urodelen liegen, soviel mir bekannt, noch keine Angaben über Zwitterbildung vor.

"Spengel will solche niemals angetroffen haben, obgleich er zahlreiche Salamander und Tritonen zerlegt und allein von Triton cristatus über 100 männliche Individuen untersucht hat."

While collecting data concerning the spermatogenesis and age of attainment of sexual maturity of *Spelerpes bislineatus*[1] I found that a series of sections through the testes of a 46 mm.

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[1] Although this species has long been called *bilineatus*, Dr. Green in his original description of it (Jour. Acad. Nat. Sc. Phila. Vol. I, Pt. II, 1818) gave it the name of *Salamandra bislineata*. With the change in generic name the masculine ending was adopted and the species is correctly *Spelerpes bislineatus*.

larva, collected in September, 1913, contained several eggs. The larva was nearly of adult proportions and presumably would have undergone metamorphosis the following summer.

Fig. 1, *B*, is from a drawing of the pair of gonads and kidneys, made with an Abbé camera before imbedding. It reveals the peculiarities of the glands when compared with Fig. 1, *A*, which is a drawing, at the same magnification, of a normal pair of testes and kidneys of a 48 mm. larva. The original of Fig. 1, *B*, being merely a hastily made outline drawing and the testes having been very small, I noticed the hermaphroditic condition only on careful study of the sections.

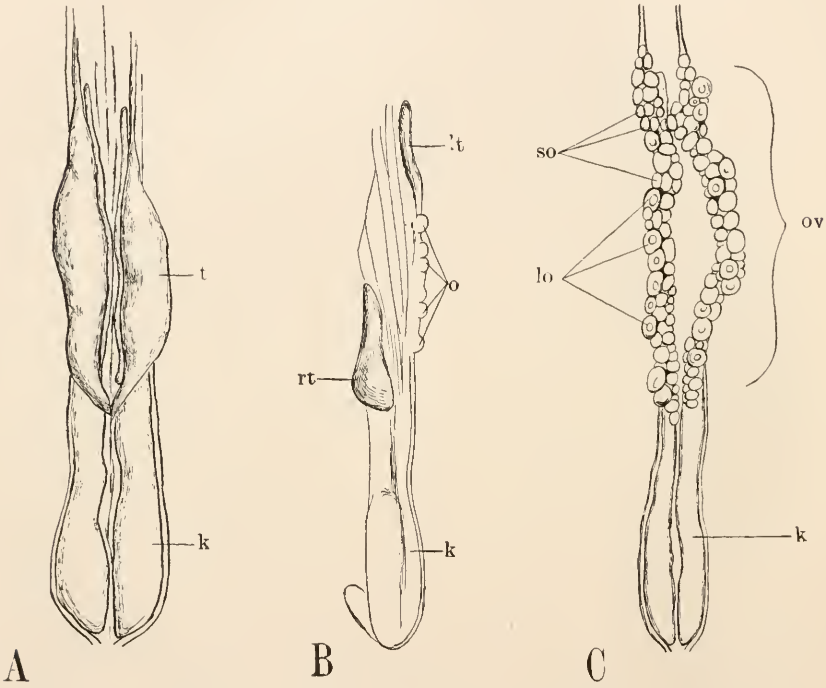

FIG. 1. *A*. Testes and kidneys of normal 48 mm. larva. *B*. Gonads and kidneys of hermaphroditic 46 mm. larva. *C*. Ovaries and kidneys of normal 46 mm. larva. These gonads and kidneys are from larvæ collected in September. Ventral view, $\times 7\frac{1}{2}$. *k* = kidney, *lo* = largest ova, *lt* = left testis, *o* = ova, *ov* = ovary, *rt* = right testis, *so* = smaller ova, *t* = testis.

A comparison of Fig. 1, *A* and *B*, shows that, in the abnormal specimen, neither testis is fully developed. The hermaphroditism is of two types. Macroscopically, the anterior part of the left gonad, which is much reduced in size, resembles the normal testis in texture, though not in shape, while the posterior region is distinctly like an ovary. The larger ova in this latter region, indicated in outline in Fig. 1, *B*, are six in number.

Fig. 1, *C*, is a camera drawing of the ovaries and kidneys of a normal 46 mm. larva, collected in September, showing the arrangement of eggs, for comparison with their arrangement in the posterior region of the left gonad of the hermaphrodite. In each normal ovary the larger ova lie approximately in two rows, one lateral and one medial. A dozen or fifteen on each side are appreciably larger than the others. These larger ones, as may be deduced from a comparison with the ova in specimens of adult female *Spelerpes*, collected in September, are of the size and state of development which indicate that they would normally have been deposited a year from the following spring as the first brood of this individual, while the next smaller ones would have been deposited two years from the following spring.

The larger ova in the left gonad of the hermaphrodite are about the size of the smaller ova in the normal ovaries, though their irregularity in shape, especially where large numbers of eggs are packed together, makes exact measurements impossible. The ova of both the normal specimen and the hermaphrodite have their long axes parallel to the longitudinal axis of the body. This diameter, as measured from sections, is .15 to .20 mm. while the shorter diameter, at right angles to the long axis, averages about .12 mm. The largest ova found in the hermaphrodite are, in development, a year behind the largest ova in a normal female having the same total length, collected at the same time, and therefore, presumably, of the same age. Beside these ova, there are oögonia in the left gonad of the hermaphrodite, similar to those in the normal ovary which have not yet begun to elaborate yolk (Fig. 2). The relation of ova to follicle cells and to peritoneum in the hermaphrodite is like that in the normal ovary.

Although smaller than the right testis, the anterior portion of the left gonad has somewhat the structure of the normal male gland. The elongated mass is composed of too few loulebs to have the usual arrangement about a collecting duct. The cells, showing no signs of division to form cysts, which is the immediate preparation for spermatogenesis, are not as far advanced in their development as the germ cells of a normal male larva several months younger than the hermaphroditic one. The left gonad shows throughout a retarded development.

The right gonad, on the other hand, although smaller than the normal testis of an individual of the same size, seems to be, in general, normal in structure and in its cellular development. However, it shows another sort of hermaphroditism. Two ova are, in this case, found in the otherwise apparently normal testis, each one completely filling one lobule, which would normally contain a large number of male cells (see Fig. 3). These ova are about two thirds the size of those found in the right gonad. All the lobules are divided into cysts, with the excep-

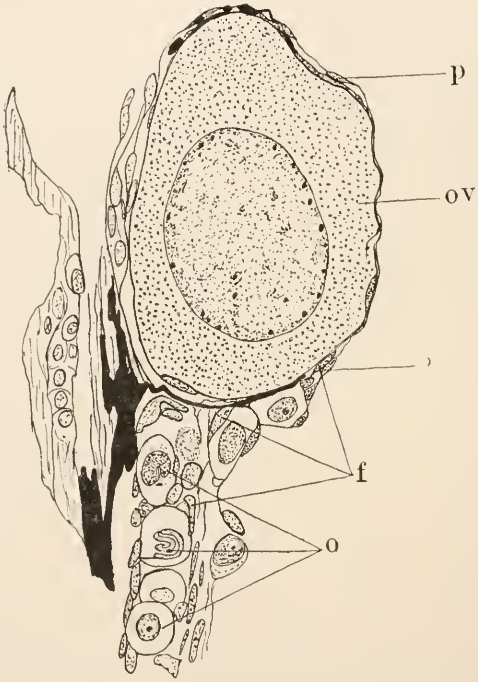

FIG. 2. Horizontal section through posterior region of left gonad of hermaphrodite, × 280. *f* = follicle cell, *o* = oogonium, *ov* = ovum, *p* = peritoneum.

tion, of course, of the two which contain a large ovum apiece. Most of these cysts are composed of spermatogonia of the last generation, having round or oval nuclei with the chromatin arranged in an irregular network. Nucleoli appear in a few cells of one section. The spermatogonia resemble Kingsbury's Figs. 1 and 2 ('02). In a few cysts, all the cells are undergoing mitosis. These do not resemble the maturation mitoses described for *Desmognathus*, and are probably spermatogonial divisions.

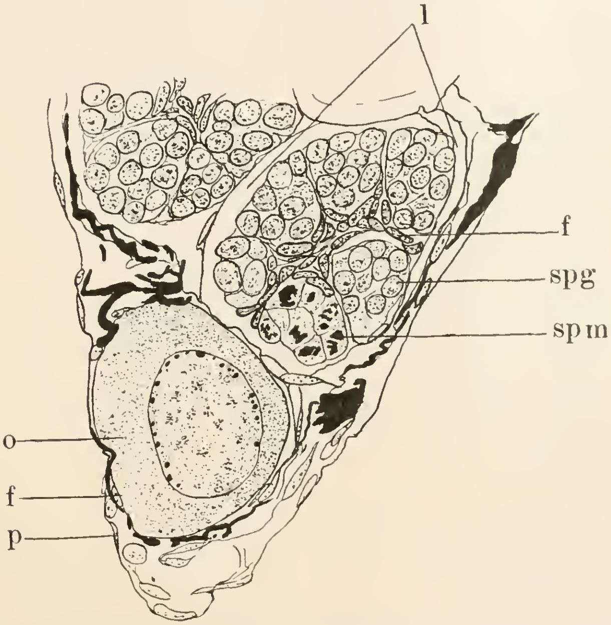

FIG. 3. Horizontal section through right gonad of hermaphrodite, × 280. *f* = follicle cell, *l* = lobule, subdivided into six cysts of spermatogonia, *o* = ovum, *p* = peritoneum, *spg* = spermatogonium, *spm* = spermatogonial mitosis.

In other cysts the nuclei are in the "contracted" condition, described by Kingsbury (Fig. 18), occasionally occurring in *Desmognathus fusca* at the beginning of the period of growth of spermatogonia into spermatocytes and more commonly, in that species, in the last generation of spermatocytes. Fig. 3 shows an egg filling one lobule and surrounded by follicle cells. In this case, as well as in the left gonad, the relation to follicle cells and to peritoneum is normal. The adjacent lobules show spermatogonia, grouped in cysts, surrounded by long follicular cells. The cells of one cyst are undergoing spermatogonial mitosis. A great deal of pigment from the peritoneum appears in this section which was cut near the surface of the testis.

The germ cells of the right gonad seem to have reached a normal degree of development. The individual, collected in September, with a total length of 46 mm., would have undergone metamorphosis during the following summer. It is probable that the male *Spelerpes* attains sexual maturity in the fall after metamorphosis. Then this individual should have reached maturity in a little over a year. The spermatogonia are just ready to commence the process of maturation. This process in *Desmognathus* takes about a year, while Kingsbury's observation that, in *Spelerpes*, regeneration of the lobule begins before the spermatozoa leave it, indicates that possibly the process of spermatogenesis requires even more time in *Spelerpes*. Then it may be supposed that these male germ cells of the right gonad would have become ripe spermatozoa at the normal time. The two ova in this gonad, however, are at least a year behind the normal development. In the left gonad, in which there is a greater abnormality, the development of both kinds of germ cells is retarded.

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NOTE ON THE NATURE AND SOURCE OF "PURPLE X."[1]

ALVALYN E. WOODWARD.

In the summer of 1913, Dr. O. C. Glaser ('14) found (1) that if a suspension of *Arbacia* sperm be boiled it turns purple, (2) that this purple color disappears if the boiled suspension be allowed to stand over night, and (3) that initiation of development in *Arbacia* eggs, either by sperm or by egg secretion, can be inhibited by the addition to the eggs of this boiled suspension *so long as it is purple.* This purple substance, he provisionally designated as "purple x."

I. Source of "Purple X."

In 1914, while working with various inhibitors of fertilization in *Arbacia*, I wished to use "purple x" and found that it did not always appear when *Arbacia* sperm was boiled. This naturally led to a search for the source of the substance. Sperm was obtained from a large number of males. Each one was rinsed in fresh water, and after the peristome was cut, placed aboral side down in a clean dry watch-glass, until the seminal fluid had been freely shed. A portion of the sperm from each animal was then mixed with filtered sea-water and examined under the microscope and a second, larger portion of each was boiled. It was found that, as a rule, the suspensions which showed fewest foreign cells—blood-cells or fragments of organs—were least likely to form "purple x." Suspensions which appeared, under the microscope, fairly free from foreign cells, could be depended upon to give a colorless filtrate when boiled.

What, then, is the impurity which produces "purple x"? By a series of experiments properly checked and repeated, I was able to eliminate successively, the filtered perivisceral fluid (serum), the blood clot which had been washed in sea-water to free it from serum, fresh fluid containing both serum and blood cells, pieces of the alimentary tract, and mesentery. If a piece of fresh mature testis be boiled in sea-water, in sea-water plus sperm, or in distilled water, a purple compound is formed. This color can also be obtained by treating the testis with strong alcohol, which, as observed under the microscope, turns testis cells from greenish brown to purplish. Whether or not this would happen with immature testes could not be determined, since the experiments were carried on during the breeding season.

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[1] From the Marine Biological Laboratory at Woods Hole and the Zoölogical Laboratory of the University of Michigan.

The sperm from one male, when examined under the microscope, showed the presence of a few small pieces of testis, and, when boiled, or treated with alcohol, turned purple. The testes were then washed in running sea-water for about three hours, during which they were occasionally pinched with forceps to help free them from sperm. When this was apparently all washed out, a portion of the testis was boiled in sea-water, without giving a purple color. Treating with absolute alcohol, boiling in distilled water, and boiling in sea-water plus fresh sperm also failed to bring out any purple tinge from pieces of the washed testes. The rest were then left standing over night in a finger-bowl of sea-water. After twenty-four hours, it was observed that they tinged the water slightly but distinctly purple.

It seems clear, then, that "purple x" arises from the fresh testis, or from a reaction between testis and sperm. It still remains to be learned whether or not it may be obtained from the ovaries also.

II. CHEMICAL NATURE OF "PURPLE X."

The best known pigment of sea-urchins is echinochrome, whose chemical reactions are well established. It may be obtained from the red blood cells by laking with distilled water, by extracting with alcohol, chloroform or ether. The neutral extracts are a cherry red, the addition of a small amount of NaOH turns them yellowish, and acidulating with HCl produces a red-yellow color (MacMunn, '85).

Echinochrome was extracted from the washed blood clot by laking with one volume of distilled water. An equal amount of "double sea-water" (sea-water boiled down to one-half its original volume) was then added so that the salt content might equal that of the solution with which it was to be compared. A series of experiments was then run in duplicate, using this echinochrome on the one hand, and "purple x" on the other. The addition of NaOH and HCl to echinochrome gave the results described by MacMunn. No visible change could be obtained in the "purple x," however, by the addition of either reagent. Thus it was established that, whatever the chemical nature of "purple x" may be, it is not identical with echinochrome.

III. Physiological Effect of Boiled Sperm.

The addition of boiled sperm suspension to eggs, in *Arbacia*, causes the jelly surrounding the egg to swell, as can be demonstrated by putting them into India ink. It also becomes more sticky. As a result of this, the eggs adhere to each other and to the bottom of the dish. When fresh spermatozoa are added, many get caught in the jelly and form "halos," but some are able to penetrate to the eggs, so that fertilization is not inhibited.

However, when "purple x" is present in the boiled sperm, fertilization by fresh sperm and auto-initiation by egg-secretion is inhibited, as shown in the following table. The percentages were all obtained by counting 200 or more eggs, and a vertical column represents eggs from the same female, treated in different ways.

	Per Cent. of Eggs Divided.						
	A	*B*	*C*	*D*	*E*	*F*	*G*
Eggs without sperm	0	0	0	0	0	0	0
Eggs + sperm	84	72	73	72.3	Polyspermy		
Eggs + boiled sperm (colorless) + fresh sperm		68	55	76.7	82		
Eggs + boiled sperm (purple) + fresh sperm	0			26.6	39		
Eggs + egg secretion + hypertonic after-treatment						43	56.9
Eggs + egg secretion + boiled sperm (purple) + hypertonic after-treatment						0	1 ±

University of Michigan.
March 17, 1915.

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ARE FUNCTION AND FUNCTIONAL STIMULUS FACTORS IN PRODUCING AND PRESERVING MORPHOLOGICAL STRUCTURE?

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Since the days of Lamarck the attempt has often been made of explaining the genesis of the morphological structure of organisms through the theory of adaptation. A special form of this theory is that of "functional adaptation" which was formulated under this name by Wilhelm Roux about 1880, and was later elaborated by that investigator in an extremely extensive and thorough manner.

The most striking organic structures are those which like the bones seem to be constructed on a definitely purposeful plan, offering the largest amount of strength with the smallest amount of material. Other organs, such as the muscles, increase in size as a result of increased function. Roux named this phenomenon "functional adaptation," while the structures underlying this principle he described as "functional structures." He made a number of exceedingly careful anatomical studies of such "functional structures." Endeavoring to explain the genesis of such seemingly purposeful structures from a purely mechanical standpoint, he found that they possessed exactly that construction which was to be expected from a mathematical calculation based on the principle of functional adaptation.

In order to make clear the development of functional adaptation, Roux fell back upon the most primitive particles of living matter. In his opinion some of these particles have been adapted to respond to functional stimuli, that is, they show a greater tendency to proliferate in the presence of functional stimuli than in their absence. Thus, those elements which were subjected to stimuli soon predominated over those which were not thus exposed.

If functional structures consisted of such particles, they would show the following characteristics: first, enlargement (functional hypertrophy) with increased stimulus; second, atrophy from inactivity upon cessation of the stimulus; third, regeneration of organs of functional structure to the condition of the original structure would only be possible in the presence of function and functional stimuli (functional regeneration); and fourth, the successful transplantation of functional structures would only be conceivable if after transplantation the organs involved were supplied with an appropriate amount of functional stimulus. The principles, therefore, necessary for an acceptance of the theory of functional adaptation are: functional hypertrophy, atrophy from inactivity, functional regeneration, and functional transplantation. (Various other factors which are similarly instrumental in this connection will be dwelt upon more extensively in a subsequent paper.) Should it now appear that these essential principles in so-called functional structures are non-existent, the theory of adaptation would then fail to adequately account for so-called functional structures.

Before giving the results of my experiments I wish to say a few words about the general value of Roux's theory of functional adaptation.

This theory at a cursory glance has many merits, and at the time when first introduced by Roux it marked a substantial advance, inasmuch as it showed that it was possible to produce highly purposeful structures through the influence of purely mechanical principles. On this basis the theory has become a material factor in connection with the investigations and studies of many workers in the realms of pathology and physiology.

Roux's theory, however, has great disadvantages, one of which is its extremely complicated and extensive terminology. It is probably owing to the extreme obscurity of the doctrine that comparatively few investigators, apart from the originator of the theory, have familiarized themselves with it and fully understood its principles. As a result of this practically the only valuable work along this line has been carried on under the direct control of Roux himself, while in contrast with the comparatively scanty publications emanating from Roux's laboratory an amazingly voluminous mass of literature has been supplied by outside writers, all of whom were under the impression that they were contributing something to Roux's theory, but who in reality had hardly grasped more than a few but imperfectly comprehended terms connected with the essential problem. These for the most part misleading publications have caused more error than progress in experimental work. I shall endeavor to prove this point in a subsequent and more extensive article.

A second disadvantage of Roux's theory is the fact that the extent of the field in which it is applicable becomes more and more restricted with the increasing number of "experimental" investigations bearing directly upon this problem. Consequently, in course of time the phenomena which are not in harmony with this principle of functional adaptation increase in number, although they may be explained, together with other phenomena, from another point of view.

However, by far the weightiest objection to the theory is that it threatens to become more and more of a stumbling block to workers who are setting out to investigate the problems of organic and inorganic life from a common viewpoint. Nowadays theories such as that of function and functional stimulus can hardly be reconciled with a chemico-physical view of the life processes; for the whole underlying principle of the theory of adaptation does not lend itself to methods of measurement. For this reason a detailed revision of the theory of functional adaptation has become necessary and will be published in a later communication.

In the present paper I shall report on the results of a few experiments which I began five years ago with the above mentioned object in view. As they are at the present moment sufficiently advanced to allow of a survey of the whole point at issue and to show that they are qualified to throw some light on the problem of functional adaptation, a preliminary discussion of these experiments may be warranted.

The experiments in question were performed on the transplanted eye of *Salamandra maculosa*. The eye of a larva was transplanted into the neck of another larva, where after a few days' partial or complete disappearance of the retina resulted, ending finally in complete degeneration. *The remarkable fact* *was that this degeneration was followed later by a complete regeneration of the transplanted eye, even in the dark.*

My experiments were conducted with the following considerations in view. The retina is one of those structures which according to Roux's definition should be called a functional structure. But in accepting this definition one need not necessarily assume that these structures are the result of functional stimulus, for such structures may have been predetermined in the embryonic stage. They develop independently of functional stimulus until they reach the third period—the so-called functional period.[1] Functional structures can only exist during this period if they are supplied with a sufficient amount of functional stimulus; otherwise they undergo atrophy through inactivity. From Roux's point of view it would appear probable that the morphogenesis of the eye of *Urodela* is only partially determined by inheritance, and in accordance with this determination it would reach the same stage of development as that attained by *Proteus anguineus*. The further development of the eye, comprising the formation of the rods and cones, that is the true functional parts, would be brought about by the penetration of the rays of light through the skin of the salamander, to its ovaries, and would therefore be the outcome of functional stimulus. Secerov[2] asserts that the skin of *Salamandra maculosa* permits of the penetration of approximately 1/173 of the quantity of light in which the animal lives.

The eyes of *Proteus*, which inhabits dark caves, must therefore remain in the primitive *Proteus* stage, according to the statement of Kammerer,[3] who believes he has demonstrated that further differentiation can only occur if *Proteus* be kept in the light. Thus, he ascribes the process of full differentiation of the eye in the case of several *Proteus* which were kept in the light, directly to the influence of function, and Roux is apparently of the same opinion. This point will be discussed by the writer in a later paper.

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[1] See, for example, Roux, "Die Entwicklungsmechanik, ein neuer Zweig der biologischen Wissenschaft," Vorträge und Aufs. über Entwickl.-Mech. d. Organismen. 1905, Heft I., p. 94, note 11. Also Roux, "Die vier Hauptperioden der Ontogenese, sowie das doppelte Bestimmtsein der organischen Gestaltungen," *Mitteilung der naturforschenden Gesellschaft*, Halle a.d.S., 1911, I., p. 1.

[2] Secerov, S., "Die Umwelt des Keimplasmas. II. Der Lichtgenus im Salamandra-Körper," *Arch. f. Entwcklungsm.*, 1912, XXXIII., 682.

[3] Kammerer, P., "Experimente über Fortpflanzung, Farbe, Augen und Körperreduktion bei *Proteus anguineus* Laur. Zgl. Vererbung erzwungener Farbveränderungen." III. Mitteil., *Arch. f. Entwicklungsm.*, 1912, XXXIII., 349.

My own observations on the transplanted eye of *Salamandra* soon convinced me that this case lends itself very well for the test of the theory of functional adaptation.

First of all I severed the optic nerve, a procedure which according to general opinion should induce permanent degeneration of the retina as a result of the eye becoming isolated from the brain. In all previous operations of this nature, where, however, the eye remained in its normal position, a reunion of the amputated stumps of the nerve took place, so that it was natural to suppose that the re-connection of the eye with the brain brought about regeneration of the retina. In my own experiments I obviated the possibility of such subsequent reunion of the eye with the brain by transplanting the eye to an abnormal position (in the neck of the salamander). But regeneration took place in spite of this fact. The point of chief interest, however, is the fact that by means of this operative measure, which, as has been demonstrated in 95 per cent. of the cases, excludes reunion of the eye with the central nervous system, the eye is permanently deprived of functional power.

It is thus obvious that in these eyes no function was possible and the experiment therefore shows that a whole series of phenomena, hitherto designated as cases of functional adaptation, require a different explanation. We will now discuss these phenomena in greater detail.

I. In about a week after transplantation of the eye into the neck of the salamander the retina had degenerated to such an extent that in many cases only the peripheral part of the retina, which was not differentiated in layers, had survived.[1] But in spite of its permanent isolation from the brain and despite the fact that the eye was permanently deprived of function, the retina from this time on began to show signs of regeneration and the transplanted eye began to receive a progressively im-proved supply of blood, so that in a comparativey short time (from 4 to 6 weeks) it had regained a perfectly normal structure.

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[1] E. Uhlenhuth, "Die Transplantation des Amphibienauges," *Arch. f. Entwcklungsm.*, 1912, XXXIII.

II. This regeneration of the transplanted eye even takes place when the organ is deprived of functional stimulus by light. A series of salamanders operated on in the above-described manner, were placed in a dark room where neither red nor white light could penetrate to their eyes; but in spite of this fact the transplanted eyes regenerated and developed a normal retina.

These experiments show that the "quality" of this process, namely, regeneration as such, is independent of any sort of functional influence. We are here dealing with a case of simple regeneration, such as is found in many organs, not with *functional* regeneration, such as we might expect to find in so-called functional structures. Of course this fact does not warrant us in entirely rejecting the theory of functional adaptation, for the possibility must not be ignored that although regeneration occurs in eyes treated in this way as a result of the agency of certain other factors, nevertheless degeneration brought about by atrophy through inactivity might follow later, as a result of the permanent lack of function and functional stimulus, a possibility which would be expected to arise according to Roux's theory.

III. But secondary degeneration as a result of atrophy from inactivity failed to occur in my experiments, even when the eyes were permanently deprived of function, as occurred in the "light" series.

IV. Degeneration similarly failed to occur in the transplanted eyes which were permanently deprived of both function and functional stimulus, namely in the "dark" series. These eyes, although severed from the brain and in permanent darkness, grew and metamorphosed simultaneously with the normal eyes of the hosts.[1]

Up to the present time I have had at my disposal preparations of eyes of the "dark" series which were preserved 15½ months after transplantation; at that time the hosts were about 21 months old, and all the structures, with the exception of the sex organs, were perfectly developed. The retinas of these transplanted eyes were found to be normal in every detail.

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[1] E. Uhlenhuth, "Die synchrone Metamorphose transplantierter Salamanderaugen (Zugleich, Die Transplantation des Amphibienauges II. Mitteil.) *Arch. f. Entwcklungsm.*, April, 1913, XXXVI., 211.

In addition to the above I have a preparation of an eye of the "light" series, which was preserved 3 1/4 years after transplantation, at which time the sex organs were also fully developed. According to Roux the eye of an amphibian should by that time already have entered the functional period, as is believed to have been proved in the case of the eye of the *Proteus*. Nevertheless, the old transplanted eyes were also found to be normal, and the functional parts of the retina, viz., the rods and cones, were present and well developed.

The above results, namely, the permanent preservation in a normal condition of transplanted eyes, prove beyond any doubt that the so-called functional structures of the eye do not undergo atrophy through inactivity, even if they are kept under extremely unfavorable conditions and are deprived of all function and functional stimulus.

This fact alone is sufficient to show that atrophy from inactivity, which is one of the fundamental postulates of Roux's theory, is by no means a phenomenon of general occurrence which takes place in all so-called functional structures permanently deprived of functional stimulus, as was supposed to be the case.

As far as regeneration is concerned, the experiments mentioned above only serve to show that regeneration in itself is independent of function and functional stimulus.

V. I am, moreover, able to demonstrate that the "quantity" of the regenerative process in the eyes, viz., the rapidity of this regeneration are not influenced by functional stimulus, viz., light.

A certain number of animals (260 in all) of both the light and the dark series were preserved at certain intervals of time and the transplanted eyes were cut in sections. Eyes preserved at equal intervals of time were then compared with each other. It was found that the transplanted eyes of the same series which had lived on the host for the same period of time may show considerable differences in the rapidity with which they undergo regeneration, even if they are subjected to equal conditions as far as functional stimulus is concerned. These differences must therefore be caused by other non-specific factors. In order to ascertain how far-reaching is the influence of light, it was necessary to determine the average rapidity of regeneration in every group of eyes of the same series and make a curve for each series. Although these curves are not yet completed, the results thus far obtained show no differences between light and dark series at all. Even the quantity of the regeneration is therefore uninfluenced by light.

From the above data we must draw the following conclusions: *Functional adaptation plays no part either in transplantation or in regeneration of the retina; nor is it a factor which determines either the quality or the quantity of these processes.*

This, of course, does not mean that regeneration or transplantation of the eye cannot be influenced at all by chemical or physical factors. On the contrary, as is shown by the differences between transplanted eyes of the same series, examined at equal intervals of time after transplantation, the quantity of regenerative processes, viz., the rapidity of regeneration are subject to variation by one or more factors. The point of importance is the fact that these factors are not connected with the specific functional stimulus, viz., light. Apparently they are the same factors which also affect the rapidity of the regenerative process of other organs. The factor concerned is probably the length of time since circulation in the transplanted eye was reëstablished.

Hitherto the regeneration of the retina has been considered as being different from the regeneration of the bones. It was supposed that a bone could only regenerate its architectural structure if in use, otherwise the result would not be a normal bone but a disorderly bony mass.

Aug. Bier,[1] however, has shown that even the bones do not regenerate as a mere indefinite mass of bone if kept without functional stimulus, but that on the contrary, in the absence of any such stimulus, they resume their original functional structure to the minutest details. In certain experiments a considerable part of a human tibia was removed. Although these bones were not exposed to any function, the tibiæ after a certain length of time assumed their normal shape and structure, but only if they were supplied with nourishment in a proper way, and if sufficient space was left for them to regenerate the missing part.

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[1] Aug. Bier, "Beobachtungen über Knochenregeneration," *Arch. f. klin. Chir.*, Dec. 1912, c, 91.

The same is true of the tendons. H. Triepel[1] showed that the tendo Achillis of a cat can regenerate only tendon tissue, irrespective of the presence or absence of functional stimuli.

Our own experiments now prove the same principle also in the case of the eye of *Salamandra maculosa;* this organ regenerates its functional structures in the absence of functional stimulus, and furthermore it retains its structure permanently, despite the permanent absence of functional stimulus. For a long time it was believed that a bone only regenerated a structureless mass of callus in the absence of function, and according to this theory it would be assumed that an eye if once degenerated would in the absence of light regenerate undifferentiated retina cells, such as we find in the normal *Proteus* eye. Nevertheless, both eye and bone regenerate the normal and fully differentiated structure, even in the absence of functional stimulus.

We have seen that in my experiments the velocity of the process of regeneration was not influenced by function; and even if this had been the case it could not be used as a proof in favor of functional adaptation. There are a number of well known morphogenetic processes, the rapidity of which can be accelerated by light, although light bears no relation to the function of the developing organ, that is to say, is not a functional stimulus. The most striking experiments made in this connection are those of J. Loeb,[2] in which he showed that the regeneration of the hydrants of *Eudendrium* is impossible in the absence of light. Nevertheless, we cannot call this a case of functional adaptation, because here light is obviously not a functional stimulus. On the other hand, development of the eyes of fish embryos cannot be prevented by the exclusion of the functional stimulus. But it is incorrect to ascribe this fact to the assumption that heredity may have fixed this character so far that development of an eye now occurs in the absence of light. For Loeb[1] has shown that it is very easy to prevent the development of these eyes by a number of different means, such as lack of oxygen, which is a non-specific, non-functional factor. Moreover, it is not necessary to point out that the influence of light on a photographic plate has never been considered to be a case of functional adaptation, although the sensitiveness of the plate to light is just as much response to a physical factor as is the regeneration of *Eudendrium* or as might be the variation in the rapidity of eye-regeneration which although not found in our experiments, might possibly have occurred.

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[1] Triepel, H., "Selbstständige Neubildung einer Achillessehne," *Arch. f. Entwcklungsm.*, Aug., 1913, XXXVII., 278.

[2] Loeb, J., "Über den Einfluss des Lichtes auf die Organbildung bei Tieren," *Pflügers Arch.*, April, 1896, LXIII., 273.

The theory of functional adaptation complicates instead of simplifying the problem. What we should emphasize is not the fact that the result of the response to light is different in the case of *Eudendrium* from what it is in the case of the regenerated eye or of the photographic plate, but the fact that these three phenomena all possess a common basis. It is obvious that all three are governed by the same laws, with which we are familiar from our knowledge of physics and chemistry; but these laws are free from such terms as function, functional stimulus, or any other stimulus, or the principle of adaptation. In order, therefore, to obtain a fertile method for attacking the problems confronting us we must constantly bear in mind the fact that the same laws expressed in the same terms can explain both organic and inorganic phenomena.

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[1] Loeb, J., "Heredity in Heterogeneous Hybrids," *Jour. of Morphol.*, March, 1912, XXIII., 1.

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BIOLOGICAL BULLETIN

STUDIES IN ARTIFICIAL PARTHENOGENESIS.

II. Physical Changes in the Egg of Arbacia.

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I. Physical Organization of the Egg.

In spite of the many researches on the sea-urchin egg very little is known of its physical make-up. Year after year the egg is used in attempts to analyze fundamental problems and various theories have been based upon experiments with it. And yet, but little is known definitely about the type of physical organization which it possesses. Is the egg essentially fluid or is it a more or less rigid jelly, is the unfertilized egg surrounded by a microscopically visible membrane or by a hypothetical film beyond the limits of vision, what indeed is the nature of the membrane which controls osmotic interchange? These are some of the questions which must be considered if our theories are to be more than mere generalizations.

The first point to be decided upon is the physical nature of the egg contents. Embryologists in the past have often observed flowings in egg cytoplasm and such observations indicate a fluid composition. This would accord with Rhumbler's demonstration of fluidity in other types of protoplasm. We might accept these results without further comment if it were not for the fact that recently there has been a tendency to regard protoplasm as a gel. Kite ('13) has in fact concluded that the protoplasm of the starfish egg is of this nature. The protoplasm of the normal unfertilized sea-urchin egg is undoubtedly in a typically fluid condition. If pressure is applied to eggs underneath a coverslip, the egg contents flow out, indeed, if the pressure is vigorous enough, the protoplasm is shot out in a long stream as from a pipette. Facts such as these are probably known to many embryologists, similar observations have been especially described by Reinke ('95) and Albrecht ('98).

If the egg is essentially a fluid mass, what is there to prevent it from diffusing through the sea-water? Two possibilities exist, either (1) The substance of the egg is as a whole insoluble in sea-water or (2) it is surrounded by a membrane insoluble in sea-water.[1] The first possibility is excluded by the fact that we know protoplasm to be in aqueous solution. We must therefore conclude that the egg is surrounded by a membrane insoluble in sea-water. Careful observation reveals the existence of a membrane around the unfertilized egg; just outside of the darker substance of the egg cytoplasm, a dim outer line can usually be made out. The faintness with which the outer margin of this vitelline membrane appears is apparently due to the fact that its refractive index is very close to that of sea-water. That the appearance of a membrane is not the result of a diffraction illusion is shown by the fact that the membrane may be isolated by pressing out the egg contents, as Herbst first found. At fertilization, as will be pointed out more fully later, it is this membrane which becomes lifted from the surface of the egg as the fertilization membrane.

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[1] The oft-made assumption of a surface film like that found at the surface of peptone solutions, etc., is really a special case of the first alternative. For such a film could only exist at a surface of discontinuity, and this could only occur at the junction of 2 immiscible fluids.

A study of the chemical behavior of the membrane gave results of interest. Dilute acids cause it to swell. When one part of normal HCl is diluted with 9 parts of sea-water the resultant solution causes a marked swelling. The membrane becomes sticky and agglutination follows.[1] Dilute solutions of nitric, butyric, and valerianic acids give similar results. As far as can be ascertained however, this acid swelling of the membrane does not result in complete solution. On the other hand dilute alkaline solutions, although they cause little if any swelling of the membrane, are quite effective in dissolving it away. In order to study the effect of alkali on the membrane, it is best to shake the jelly off the eggs first, as this often becomes saturated with the $Mg(OH)_2$ precipitated by the alkali, and obscures the result. Eggs in 50 c.c. of sea-water plus 2.5 c.c. *n*/10 NaOH soon become sticky; they cling to the bottom of the dish and to each other. Soon the exterior surface of the egg becomes rough, it is evidently no longer surrounded by a membrane (and it is only prevented from diffusing through the sea-water by coagulation). Many salts exert a swelling effect on the membrane, and in some cases this is accompanied by a complete solution of the membrane. When eggs are dropped into 0.55 *N* NaI, the swollen membranes are very apparent after 10 or 15 minutes have elapsed. In 40 minutes almost all of the eggs are no longer surrounded by a membrane, the periphery of these eggs instead of being smooth, now presents a roughened appearance. It is quite evident that the protoplasm is naked and that the membrane has been completely dissolved away by the sodium iodide. This behavior of the membrane towards acids, alkalis, and salts, indicates its protein nature.

That it is not a lipoid is evident from the fact that it is insoluble in any of the ordinary lipoid solvents. It might however contain an admixture of lipoids. This is rendered improbable by the following line of evidence. As was pointed out above, the almost invisible character of the membrane indicates that its refractive index is very close to that of sea-water. Now it is well known that many proteins have a refractive index close to that of sea-water; on the other hand, lipoids have a considerably higher refractive index. As the refractive index is an additive function of the constituents of a mixture, the presence of lipoids in any abundance would make it impossible for the refractive index of the membrane even to approach that of sea-water. Hence no great admixture of lipoids can be present. More direct evidence of the absence of any appreciable quantity of lipoids is also available. The membrane was tested with a Scharlach R solution such as recommended by Herxheimer. In order to render them more visible, the membranes were made to swell by placing the eggs in 15 c.c. sea-water plus 10 c.c. 2.5 *N* NaCl. To a drop of Scharlach R solution on a slide was added a drop of egg suspension. The vitelline membrane remained hyaline and unstained, whereas the egg cytoplasm itself was colored red. The Scharlach R solution used in this test was a saturated solution of the dye in equal parts of acetone and 70 per cent. alcohol.

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[1] Loeb ('08) described agglutination in HCl.

If the egg contents be made to flow out from the membrane or if the egg be cut or shaken into fragments, a new membrane immediately forms around the momentarily naked protoplasm.[1] Such a membrane has the same chemical properties as the vitelline membrane. The acids and salts which produce swelling in the latter, cause it to swell also. The immediate production of a protein membrane about these egg fragments must be regarded as similar to the formation of precipitation membranes like those studied by Traube and Quincke. Evidently, some protein contained in the egg is precipitated on contact with the outer sea-water. It is probable that this protein is, in the interior of the egg, prevented from coagulation by the presence of a protecting colloid. At the outer surface of the egg, the membrane-protein is coagulated by direct contact with sea-water. Support for this view is found in the fact that the presence of some colloids (*e. g.*, egg albumen) in the sea-water, causes the membrane to take up water and swell. Since the vitelline membrane behaves toward reagents in the same manner as does the membrane on egg fragments, we are justified in regarding it too as a precipitation membrane. The most noteworthy feature of the precipitation membranes of Traube and Quincke is their semipermeable (or partially semi-permeable)[1] character. We should therefore expect the vitelline membrane to exhibit semipermeable properties, and in this way to govern osmotic interchange. When the egg is caused to shrink in a hypertonic solution, the vitelline membrane shrinks with it. This has been observed in a great variety of hypertonic solutions. For such a shrinkage, there are 3 possible types of explanation. The inward tension may be the result of a force arising from (1) the substances within the membrane, (2) the membrane itself, or (3) something immediately outside of the membrane.

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[1] O. and R. Hertwig in their "Untersuchungen zur Morphologie und Physiologie der Zelle," Heft 5, 1887, first observed the elevation of membranes on egg fragments. (This observation was also repeated by Ziegler, Arch. f. Ent. Mech., VI., 249 (1898), by Moore, Univ. of California Publications in Physiology, IV., 89 (1912).

Physico-chemically the possibilities, as I see them, are (1) The fluid interior of the egg is coagulated by the various hypertonic solutions, shrinks as a result and pulls the membrane with it. (2) The vitelline membrane as a result of its semipermeability[2] is responsible for the shrinkage. (3) There is an invisible semipermeable membrane outside of the vitelline membrane. As there is nothing to warrant the assumption of any semipermeable membrane outside of the vitelline membrane, it seems scarcely necessary to discuss the third possibility. The first possibility is essentially the position maintained by M. Fischer ('10). He regards the passage of water into and out of cells as due primarily to the attraction of the interior colloids for water. According to this view endosmosis, such as occurs in hypotonic solutions, would be the result of the taking up of water by colloids within the cell. That the process of water absorption is by no means dependent on the affinity of the egg colloids for water is conclusively proven by the fact that influx of water leads not to transformation of gel to sol, as we would have to suppose, but on the contrary such an influx transforms the egg protoplasm from the sol to the gel condition. The experimental evidence in support of this fact is given on p. 195. *Water absorption by the egg is indeed correlated with a loss of water on the part of the egg proteins.* The passage of water into and out of the cell can not therefore be due to changes in the water content or aggregation state of the egg proteins, and the first possibility is unable to account for the facts. Only the second possibility remains, *i. e.*, that the vitelline membrane acts as a semipermeable membrane and controls osmotic intercourse. On the basis of this view many facts are understandable, which can be explained in no other way. This will, I think, be demonstrated in the course of the argument.

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[1] The existence of an absolutely semipermeable precipitation membrane (*i. e.*, one which prevents the passage of all substances in solution, but permits the passage of solvent) is extremely doubtful; *cf.* for example Quincke '02. The fact that a substance may penetrate a membrane and yet exert considerable osmotic pressure against it, has often been neglected by biologists. To assume that because a substance passes through a membrane it can exert no osmotic pressure against it, is just as foolish as to assume that the air in an air-bubble exerts no pressure on the film of water surrounding it.

[2] Using the term in its broadest sense.

The plasma-membrane of a cell is defined as the membrane which governs its osmotic intercourse. According to this definition, *the vitelline membrane is the plasma-membrane of the Arbacia egg cell.* Hitherto no one has either described a plasma membrane, or studied directly the properties of one. Although he often uses the concept of such a membrane, Lepeschkin ('11) admits that the actual structure is "zurzeit unbekannt." Loewe, ('13) in referring to the plasma-membrane, says: "Allein weit davon entfernt, dasz auch nur ihre Existenz irgendwie sichergestellt wäre, sind auch über die Beschaffenheit dieses hypothetischen Gebildes die Meinungen so zahlreich wie die Möglichkeiten." Fischer ('12) is indeed of the opinion that "the entire conception of an osmotic membrane about cells is an impossibility."

The plasma-membrane of the *Arbacia* egg is a protein gel. As such, it possesses a certain degree of rigidity. Suppose a hypothetical system completely surrounded by an extremely rigid semipermeable membrane.[1] If such a system were placed in a concentrated solution no exosmosis could take place, for if the membrane were perfectly rigid, there could be no removal of solvent from the system without the production of a vacuum. But the membrane would be subjected to a considerable pressure, which would tend to make it rearrange its particles in such a fashion that the volume enclosed within it might be lessened. Whereas an extremely rigid membrane would resist such forces, one with only a certain degree of rigidity would yield (in the case of sufficient pressure), and exosmosis would be possible. Thus osmosis in an enclosed system depends, to some extent at least, on the rigidity of the confining membrane. These conclusions apply in some measure to the sea-urchin egg, for the vitelline membrane possesses a slight degree of rigidity. Most salts in hypertonic solution cause the membrane to absorb water and swell, the gel becomes less stiff, and the particles of the membrane can more readily rearrange themselves. Thus shrinkage of the egg is favored. We should in fact expect that hypertonic solutions of salts which cause membrane swelling would be more effective in causing shrinkage than those which do not.

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[1] Possibly this is the case in the *Fundulus* egg.

Direct evidence of this fact is difficult to obtain without introducing complications. One might compare the shrinkage of the egg in two solutions of equal osmotic strength, one of which causes membrane swelling and the other of which does not. But we have no means of knowing when two solutions are of equal osmotic power. Vant Hoff's law does not apply with sufficient accuracy to warrant its use,[1] and even if two solutions could be obtained which were isosmotic towards a given membrane, they would not necessarily be isosmotic towards the plasma-membrane of the *Arbacia* egg, which is very probably only partially semipermeable. Fortunately there is a way out of the difficulty. I found that when 2.5 *N* NaCl was added to sea-water in the proportion of 8 parts by volume of the former, to 50 of the latter, the resulting hypertonic solution would usually cause membrane swelling when freshly prepared, but would in large measure lose this power after it had stood for some time. Thus one can obtain two solutions of identical strength, one of which produces a softening effect on the membrane, the other lacking this effect. A number of experiments showed that in every case the eggs shrank more in the solution which caused membrane swelling than in the solution which left the membrane with its original rigidity. Two sample experiments are recorded here. To save time the term "NaCl hypertonic sea-water" is used to designate 50 parts (by volume) of sea-water plus 8 parts of 2.5 *N* NaCl.

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[1] *Cf.* Findlay, "Osmotic Pressure," London, 1913 (Chapt. IV.).

August 21. Two small stender dishes, *A* and *B*, were used. *A* contained 29 c.c. of NaCl hypertonic sea-water made up on August 18, *B* contained 29 c.c. of NaCl hypertonic sea-water freshly prepared. At 10:04 A.M., 5 drops of egg suspension were added to *A*, and a similar amount to *B*.

The following measurements of egg diameters were made at the times indicated. In making these measurements a Spencer movable scale micrometer was used. It was not found possible to obtain any very great accuracy in the use of this instrument. If the eggs are not subjected to pressure of the coverslip, they tend to move their position slightly. After various attempts I reached the conclusion that the use of the movable scale was inadvisable, especially as the usual difficulties of focusing made very accurate measurements out of the question. Accordingly the measurements were made with the scale stationary. No great claim for accuracy is therefore made, but the error is not over one micron. Fortunately the difference between the diameters of the two sets of eggs is markedly greater than the experimental error of the method. The measurements were made at a magnification of about 650 diameters.

DIAMETERS OF EGGS IN *A*.

70 μ	×	70.5 μ	at	10.25 A.M.
69	×	71	at	10.28 A.M.
69	×	70	at	10.29 A.M.
67.5	×	69	at	10.30 A.M.
69	×	69	at	10.51 A.M.
68	×	71	at	10.53 A.M.
68	×	71	at	10.55 A.M.
69	×	69	at	10.57 A.M.
68	×	69	at	10.58 A.M.

Average 69.2 μ.

DIAMETERS OF EGGS IN *B*.

66 μ	×	66 μ	at	10.32 A.M.
67	×	68	at about	10.35 A.M.
66	×	67	at about	10.35 A.M.
66	×	66	at about	10.35 A.M.
66	×	66	at	11.15 A.M.
70	×	70.5	at	11.17 A.M.

The above egg showed a completely unswollen membrane, and so should be excluded from the experiment.

63.5	×	65	at about	11.20 A.M.
67	×	67.5	at	11.24 A.M.

Average 66.2 μ (egg with unswollen membrane excluded).

The eggs used in the above experiment were all from a single female. The normal untreated eggs of this female were practically spherical and measured 74.5μ, 75μ, 75μ, 74μ, 75μ, 75μ, 75μ, 75μ, 75.5μ, 75μ. They thus possessed an average diameter of 75μ and were practically all of the same size. The experiment shows that the eggs in *B* with swollen membranes shrank more than did the eggs in *A* with unswollen membranes.[1] The average decrease in diameter of the former was about 9μ, of the latter about 6μ. The difference was also constant, for the largest egg with swollen membrane was smaller than the smallest egg with unswollen membrane.

August 23. Two small stender dishes were used. *A* contained 29 c.c. of "NaCl hypertonic sea-water," which had been made up on August 18 (at 11:30 P.M.). *B* contained 25 c.c. of sea-water. At 9.55 A.M., 4 drops of an egg suspension were dropped into *A* and the same amount in *B*, and then 4 c.c. 2.5 *N* NaCl were added to *B*, so that this solution became "NaCl hypertonic sea-water." The diameter of the eggs was determined as in the previous experiment. At 10:25 it was noticed that some of the eggs in *A* were beginning to acquire swollen membranes, so that only a few measurements were made after this time, and only those eggs with unswollen membranes were selected.

Diameter of Eggs in *A*.

65 μ	×	65 μ	at	10.12 A.M.
65	×	66	at	10.13 A.M.
67.5	×	70	at	10.14 A.M.
65	×	70	at	10.15 A.M.
65	×	68	at	10.16 A.M.
63.5	×	70.5	at	10.17 A.M.
65	×	66	at	10.18 A.M.

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[1] This result was obtained in spite of the fact that membrane swelling always tends to produce an *increase* in egg volume. Whenever membrane swelling occurs in isotonic solutions, the egg rapidly increases its volume, it cytolyzes. The cause of this cytolysis resulting from membrane swelling will be considered later.

Diameter of Eggs in *A*.

65	×	68	at	10.19 A.M.
65	×	65	at	10.20 A.M.
66	×	66	at	10.26 A.M.
66	×	69	at	10.30 A.M.
66	×	68	at	10.31 A.M.
66	×	67	at	10.32 A.M.

Average 66.5 μ.

Diameter of Eggs in *B*.

63 μ	×	63.5 μ	at	10.04 A.M.
63	×	63	at	10.05 A.M.
64	×	65	at	10.06 A.M.
63	×	64	at	10.06 A.M.
64	×	65	at	10.07 A.M.
62	×	65	at	10.08 A.M.
63	×	63.5	at	10.08 A.M.
62	×	62	at	10.09 A.M.
63	×	63.5	at	10.10 A.M.
63.5	×	64	at	10.11 A.M.
64	×	65	at	10.34 A.M.
63.5	×	63.5	at	10.35 A.M.
63.5	×	66	at	10.36 A.M.
63.5	×	65	at	10.37 A.M.
63	×	63.5	at	10.38 A.M.
62	×	66	at	10.39 A.M.
64	×	64	at	10.40 A.M.
63.5	×	65	at	10.41 A.M.
63.5	×	64	at	10.41 A.M.
63.5	×	65	at	10.42 A.M.

Average 63.7 μ.

In this experiment also only eggs from a single female were used. The untreated eggs measured 72μ x 73μ, 72μ x 73, 71μ x 73μ, 71.5μ x 74μ, 71.5μ x 73μ. Considering the eggs as spherical, their average diameter was 72.4μ. Thus on the average, the diameter of the eggs in *A* decreased 5.9μ, whereas the diameter of the eggs in *B* decreased 8.7μ. Thus exosmosis was much more pronounced in the solution which caused membrane swelling than in a solution of equivalent concentration in which this effect was lacking.

II. Cortical Changes.

A. Membrane Elevation and Membrane Swelling.

When the sea-urchin sperm comes in contact with the egg, almost immediately the vitelline membrane is lifted away from the egg surface. This is the well-known process of membrane elevation, or, as it is usually spoken of in this country, membrane formation. During elevation, the membrane under normal conditions does not undergo any evident increase in thickness. The inner border of the vitelline membrane is often not very plainly visible. In order therefore to estimate the thickness of the membrane after elevation, it is convenient to compress the eggs gently. The egg then becomes pushed out against the vitelline membrane, in some directions at least, and the distance between its outer border (*i. e.*, the hyaline layer, see below) and the outer boundary of the vitelline membrane is a measure of the greatest possible thickness of the membrane. Under such conditions, high power examination showed that the thickness of the elevated membrane is approximately the same as that of the unelevated membrane. Quantitative measurements were not found to be practicable.

Moreover, after elevation the membrane still retains the same chemical properties that distinguished it before fertilization. In dilute HCl it swells rapidly, and soon becomes sticky. NaI also induces the elevated membrane to swell, much as it did before fertilization.

After the vitelline membrane has been lifted from the egg surface, a new membrane appears around the cytoplasm. This structure has received an unusually large number of names; of these I shall use the term "hyaline layer." It seems reasonable to conclude that its formation depends on the same precipitation reaction which produces the membrane about egg fragments, and which is probably also responsible for the vitelline membrane. This conclusion is supported by the fact that it shows semipermeable properties.

The process of membrane swelling has often been confused with that of membrane elevation. This is perhaps due to the ambiguity of the term membrane formation. It has been the custom to apply the term whenever the observer notices something at the egg surface which he did not see at the beginning of the experiment. But membrane swelling and membrane elevation are two very different processes and are usually easily distinguishable under the microscope. The elevated membrane appears as a thin membrane at some distance from the egg surface, which is now bounded by the above-mentioned hyaline layer. On the other hand, the swollen membrane appears as a homogeneous layer surroundng the egg, a layer in which neither the inner boundary of the vitelline membrane, nor the outer boundary of the hyaline layer, make their appearance. In addition to the purely morphological differences, there are other distinguishing features. Among these may be mentioned the fact that swollen membranes are always sticky, and as a result, eggs with such membranes tend to agglutinate. Normal elevated membranes are never sticky. Another criterion depends on the fact that elevated membranes collapse when placed in a solution of egg albumen (or other colloid).[1] Swollen membranes are of course unable to collapse.

B. Permeability Changes in the Vitelline Membrane.

The elevated membrane is known to be readily permeable to electrolytes.[2] Hence, since it offers considerable resistance to their passage before elevation, it must undergo a change in permeability at some stage in the process. An attempt was made to determine if this increase in permeability took place before or after membrane elevation. In the first case, it might be considered as causally related to the process, and R. Lillie has in fact suggested that the cause of membrane elevation is an increased permeability of the "plasma-membrane." Experiments, however, have shown that *the increase in permeability follows rather than precedes membrane elevation.* Immediately after elevation, the membrane is still more or less impermeable to electrolytes. The following experiments show this to be the case:

August 21, 1913. Eggs from a single female were washed twice and then gathered into about 10 c.c. of sea-water at the bottom of a small beaker. Four or five drops of diluted sperm were then added, and the beaker shaken. At intervals of 1, 2, 3, 4, 5 minutes after insemination, the eggs were removed with a pipette and dropped into Syracuse dishes filled with 2 *M* $MgCl_2$. Just two minutes after insemination, a necessarily hasty examination of the eggs in the beaker showed that all the eggs had well-elevated membranes at this time. On the other hand, eggs removed from the beaker at the same time and placed in 2 *M* $MgCl_2$, upon later examination, showed no signs of an elevated membrane, and the membrane had evidently been pushed back against the egg. In the following table, the fractions in the second column indicate the proportion of eggs which showed the membrane elevated in the various Syracuse dishes. In each case, the numerator denotes the number of eggs with membranes elevated, the denominator the total number of eggs counted.

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[1] It is only some few minutes after elevation that this collapse can be produced by a colloid. *Cf.* p. 162.

[2] *Cf.* Loeb, "Artificial Parthenogenesis and Fertilization," p. 208.

Minutes after Insemination Before Transfer to 2 *M* $MgCl_2$.	Membranes Elevated (Free from Egg).
1	0/50
2	0/50
3	1/50
4	30/50
5	49/50

In the case of the eggs transferred to the $MgCl_2$ solution three minutes after insemination, some of the membranes were not completely collapsed, but one, two, or even several small globular expansions could be detected at the egg surface.

This experiment was repeated on August 22, 1913, with almost identical results. In this case the eggs were transferred to 2 *M* $MgCl_2$ at intervals of 2, 3, 4 minutes after insemination. At 1½ minutes after insemination hasty observation showed all the eggs to have well-elevated membranes. Thus the eggs were placed in the magnesium chloride solution after membrane elevation had occurred. Nevertheless, as the following table shows, the eggs removed to $MgCl_2$ two and three minutes after insemination, showed no membranes free from the egg.

After 2 minutes,	0/50	with membranes free from egg.
3	1/50	
4	13/50	

These experiments were also confirmed in the summer of 1914. I have interpreted the results as indicating that after elevation the membrane still remains impermeable to $MgCl_2$

(or as would no doubt be a more exact expression of fact, the membrane still offers sufficient resistance to the passage of $MgCl_2$ so that this salt exerts osmotic pressure against it). Indeed after being collapsed by the $MgCl_2$, the previously elevated membrane behaves as a semipermeable membrane and shrinks with the egg.

That the membrane is still "semipermeable" shortly after elevation is also shown by another series of experiments. It was found that the well-known collapse of the elevated membrane in solutions of egg albumen, did not take place immediately after elevation.

In an experiment of July 1, 1914, eggs were inseminated in a Syracuse dish at 9:43 A.M. Two drops of egg suspension were then placed in various Syracuse dishes containing 10 c.c. of 1 per cent. egg albumen[1] in sea-water, at intervals of 1, 3, 6, 12½ minutes after insemination. In the case of the eggs placed in the albumen solution 1 and 3 minutes after insemination, the membrane was well elevated and had suffered no collapse. On the other hand, when the eggs which had been placed in the albumen solution 6 and 12½ minutes after insemination were examined, their membranes were found to have been bent back and collapsed. This experiment was repeated a number of times, similar results being obtained in each case.

These surprising results find an easy explanation on the ground that the membrane is still partially permeable shortly after elevation. Because of this semipermeability it is subjected to the pressure of the electrolytes contained within it. Only when the membrane loses its semipermeable properties does this pressure cease and only then can the albumen cause its collapse. The albumen seems to exert a protective effect on the membrane and to prevent increase of permeability. Thus, when eggs were placed in albumen 1 minute after insemination, their membranes were found to retain their semipermeability and they collapsed upon being placed into 2 *M* $MgCl_2$ 14 minutes after insemination.

From these experiments we can, I think, conclude that increased permeability follows upon, rather than precedes, membrane elevation. At a later point, in discussing what I believe to be the true explanation of membrane elevation, I shall endeavor to point out a reason for this sequence.

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[1] Kahlbaum's crystallized egg albumen was used. The solution was filtered.

C. Theories of Membrane Elevation.

The problem of membrane elevation is quite distinct from that of segmentation, which is the central theme of the study of artificial parthenogenesis. No method of producing membrane elevation ordinarily results in more than one or two per cent. of segmentations, and many methods usually result in no segmentations whatsoever (*e. g.*, chloroform, urethane). Moreover, some of the best methods for producing segmentation do not involve membrane elevation: thus hypertonic sea-water never results in an elevation of the membrane.

Of late Loeb has been of the opinion that membrane "formation," involves the swelling of some substance at or near the egg surface. To quote from his book[1] "a colloidal substance which lies below the surface layer of the unfertilized egg or is secreted from the egg, suddenly swells by absorption of sea-water. In the typical case of membrane formation this swelling results finally in a complete liquefaction of the colloid. In other cases the swelling is less complete and the formation of a gelatinous film results." Loeb thus regards the process of membrane formation as due to the swelling of a colloid, concerning the position of which he is not quite clear. On page 213 of the same book he says it is in the cortical layer. Finally, if the egg is left too long a time in a solution which causes membrane formaton, not only a colloid at the periphery, but colloids throughout the egg swell, and cytolysis results.

In the discussion of Loeb's theory, I shall assume that he uses the word "swell" in its colloid-chemical sense, *i. e.*, to denote the absorption of a liquid (in this case water) by a gel. Of course any less specific use of the term would rob the theory of all theoretical significance. Briefly, the objections to the swelling theory, as upheld by Loeb, are:

1. All the reagents which cause membrane elevation can scarcely induce the swelling of any one colloid. Loeb claims

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[1] *Loc. cit.*, p. 210.

that some of the "membrane-forming substances" cause the egg membrane of the Mollusk *Lottia* to swell, but he admits that this is not true of all of them. Some seem to cause swelling and final liquefaction of the chorion or jelly of the sea-urchin egg, but here again all are not effective, and I can suggest chloroform as an exception. In fact it must be a strange colloid which can be made to absorb water by such reagents as distilled water, alcohol, chloroform, toluol, picric acid.

2. It is difficult to conceive of the location of the swelling colloid. The egg has been shown to consist essentially of a more or less rigid membrane surrounding a mass of fluid contents. Evidently the latter can not swell, as only gels possess this property. It is also demonstrable that the outer vitelline membrane itself does not swell in the case of true membrane elevation, for it can scarcely be doubted that the vitelline membrane undergoes an increase rather than a decrease of rigidity after elevation. And swelling is always correlated with a decrease in rigidity on the part of the gel. It is difficult to understand how Loeb seeks to explain by swelling what he regards as the *formation* of a more or less rigid membrane.

3. Cytolysis results not in colloidal swelling and liquefaction, but in coagulation. Loeb considers membrane elevation and cytolysis as due to the same processes. He says:[1] "Substances like benzol, saponin, etc., can cause both membrane formation and cytolysis. The first of the two is produced when they have time to affect only the surface of the egg; cytolysis is produced when their effect extends to the deeper layers of the egg . . . the greater the fraction of the egg which comes under the effect of the membrane-forming reagents, the greater the amount of colloid that must be liquefied." Loeb thus states explicitly that the membrane-forming reagents "liquefy" the colloids of the eggs, and that this effect in the case of cytolysis extends into the interior. Now it is a fact that the membrane-forming reagents, far from producing liquefaction, have an exactly opposite effect upon the colloids of the *Arbacia* egg. Instead of transforming a solid mass to a more fluid state, what they really do is exert a solidifying or rather a coagulating effect

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[1] *Loc. cit.*, p. 213.

upon the egg colloids. The actual evidence in support of this statement will be given at a later point (p. 196).

In the first paper of this series, after showing that all substances which produce membrane elevation cause a lowering of surface tension, I proposed a theory of membrane-elevation based on a simple consideration of the forces in equilibrium at the vitelline membrane. At the time I was not yet aware of the semipermeable nature of this membrane, and hence in discussing the various forces acting upon it, I did not consider osmotic forces as being involved. The theory therefore requires slight modification in the light of this new fact. Let us suppose in Fig. 1 that the shaded area is the vitelline membrane. The arrows represent the forces acting upon it. Arrow *A* directed outward indicates the outward force due to the osmotic pressure of the dissolved substances within the membrane. Were gels present in the egg we should also have to add the swelling pressure of these. Directed inward are two arrows, arrow *B* is the force due to the osmotic pressure of the salts outside the membrane, arrow *C* is the radial component of the surface tension of the membrane. As the membrane is slightly thicker than twice the range of molecular action, it is really composed of two surfaces of surface tension, *i. e.*, an outer surface of contact with the sea-water and an inner surface of

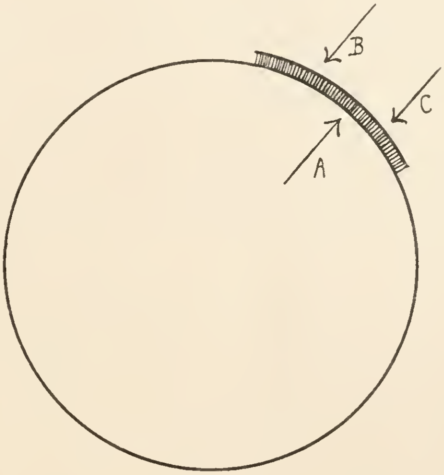

Fig. 1.

contact with the egg contents. Both surfaces are solid-liquid surfaces, and as such no doubt possess a high surface tension.[1] The arrow *C* represents the sum of the radial components of the tension of both inner and outer surfaces of the membrane. Suppose now that a substance which lowers the surface tension of water, is added to some eggs in sea-water. By what is known as the Gibbs-Thomson Law it will tend to accumulate at the surfaces of the vitelline membrane. This will result in a lowering of the surface tension of the membrane.[2] As a result equilibrium no longer exists, the force pushing outward is now stronger and the membrane is lifted away from the egg surface. Most of the egg proteins do not follow the membrane as it is lifted away, but remain in their original position. This is due to the fact that they diffuse much less readily than the salts, which can be thought of as pushing out the membrane. Around the mass of egg proteins a membrane is rapidly formed. As previously pointed out, this "hyaline layer" is to be regarded as similar to the precipitation membranes formed on egg fragments.

As a result of elevation, the vitelline membrane is no longer in contact with the egg cytoplasm. It is, accordingly, now exposed on both of its faces to a solution which has the power of coagulating it. It might be expected that the process of gelatinization or coagulation be carried a step farther, and that the membrane become more rigid. This increased coagulation, if I may so speak of the process,[1] results, as is typical for such cases, in an increase of refractive index, and the optical image of the membrane becomes more clearly defined. It may also result in an increased permeability of the membrane, for Quincke ('77) claims that precipitation membranes lose their semipermeable properties on becoming rigid.

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[1] The surface tension of a solid-liquid surface has never been accurately determined, but there are various reasons for considering it the seat of an exceptionally high tension. For the similar case of a solid-gaseous surface Freundlich ("Kapillarchemie," p. 90) following Quincke states that "die Oberflächenspannung flüssig-gasformig steigt allgemein mit sinkender Temperatur. Wenn nun die Flüssigkeit stetig in einen amorph-festen Korper—eine Flüssigkeit mit sehr groszer innerer Reibung—übergeht, musz man auch annehmen, das die Oberflächenspannung bestehen bleibt, ja das sie zunehmend gröszere Werte erhält wenn sie sich auch wegen der groszen Zähigkeit nicht äuszern kann." With the aid of a formula derived by Wilh. Ostwald, Freundlich calculates the tension of solid-liquid surface $BaSO_4$-water to be several thousand dynes per cm., an enormous value. In his wonderful treatment of capillarity Gibbs devotes a long section to the discussion of solid-liquid surfaces (Gibbs, Collected Papers, I., 314–331), and derives various equations for them. In discussing the surfaces of the membranes, I have for the sake of simplicity not considered the presence of the jelly of chorion, which surrounds the egg. This is so diffuse a gel that it no doubt [has little if any effect. In fact, after its removal (by shaking), the eggs behave just as they did before.

[2] Gibbs, *loc. cit.*, p. 274: "Now the potential of a substance which forms a very small part of a homogeneous mass certainly increases, and probably very rapidly, as the proportion of that component is increased. (See (171) and (217).) The pressure, temperature, and the other potentials, will not be sensibly affected (see (98)). But the effect on the tension of this increase on the potential will be proportional to the surface-density, and will be to diminish the tension when the surface-density is positive (see (508))." The numbers refer to equations. When a substance accumulates at a surface, its surface density is by definition positive.

On the basis of the above theory, it is evident that when the surface tension of the membrane drops below a certain limiting value, elevation occurs. The determination of this value is at present impossible. It might be thought that the determination of the surface tension of the various membrane-elevating solutions at their surface of contact with air, would throw some light upon the matter.[2] But the lowering effect of a substance on a solid-liquid (or liquid-liquid) surface tension can not be measured by the effect the same substace produces on a gas-liquid surface. The chief reasons for this are: (1) Adsorption of added substances plays a very decided rôle in the case of the solid-liquid surface, different solids of course showing different degrees of selective adsorption, (2) A solid-liquid surface is not subject to the action of evaporation, as is a gas-liquid surface. This is of especial importance if the substance which lowers surface tension is volatile. In discussing films like those of bubbles, Gibbs[3] says: "But when a component which greatly diminishes the tension of the film although forming but a small fraction of its mass (therefore existing in excess at the surface), is volatile, the effect of evaporation and condensation may be considerable, even when the mean value of the potential for that component is the same in the film as in the surrounding atmosphere."[4] Thus chloroform in water is quite effective for membrane elevation, but such a solution of chloroform has a surface tension against air just slightly below that of pure water.[1] The great volatility of chloroform explains its inability to lower markedly the tension at the water-air surface; it is unable to accumulate in the surface film. But it can readily accumulate at a solid-liquid surface and here it no doubt produces a marked lowering of surface tension. It follows that, although by a measurement of the air-water surface tension we can determine qualitatively whether a substance lowers the surface tension of the membrane or not, any truly quantitative measurements are impossible.

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[1] It might also be designated as "loss of water."

[2] Czapek ('11) has thus endeavored to draw conclusions as to the surface tension of the plasma membrane of plant cells by measurements of air-liquid surface tensions.

[3] *L. c.*, p. 310.

[4] A condition which would at least be approximated if the film were in equilibrium with its vapor in an enclosed chamber.

In order to show qualitatively that a substance lowers the surface tension of the membrane, it is only necessary to make certain that its solution in water has a lower surface tension than the pure solvent. The theoretical basis for this assertion has already been pointed out. There is also another case to be considered. Some substances exert a liquefying effect upon the vitelline membrane. The liquefaction of a gel no doubt results in a considerable lowering of its surface tension.[2] Ordinarily, however, the liquefying effect is a slow one, and in such cases, membrane elevation does not occur. For if the surface tension of the membrane is lowered only very slowly, the egg proteins have time to follow the membrane as it is pulled outwards. The result is an increase in egg diameter; the egg cytolyzes. Thus when the surface tension of the vitelline membrane is slowly lowered, cytolysis follows directly.

On the other hand, when it is rapidly lowered membrane elevation takes place first and cytolysis follows after a short interval. After the vitelline membrane is elevated, it loses its semipermeability and the hyaline layer takes its place as the plasma-membrane of the egg cell (cf. p. 159). The same forces act on this membrane as were found to act on the vitelline membrane prior to its elevation. Hence when its surface tension is lowered, since the osmotic pressure within the egg is no longer completely counterbalanced, the hyaline layer tends to become pushed outward. But the hyaline layer appears to be more closely adherent to the egg proteins[3] so that they are pulled out with it and the entire egg increases in diameter. According to this view cytolysis (when not directly an osmotic phenomenon) is in every case due to a lowering of the surface tension of the plasma membrane. This produces an influx of water into the cell.

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[1] Czapek ('11), p. 35.

[2] *Cf.* note p. 166.

[3] Probably because of a slight cortical coagulation.

Various other explanations of cytolysis have been attempted. It has been regarded as due to the swelling of the egg proteins. This is an incorrect assumption, as I have already pointed out that the egg proteins, instead of becoming swollen, are coagulated in the cytolyzed egg (see pp. 195–198). It might also be considered as the result of a change in the plasma-membrane, of such a sort that this membrane would become readily permeable for salts though still impermeable for colloids. The unopposed osmotic pressure of the egg colloids would then push out the plasma membrane and the egg would increase its diameter. But such an explanation can not hold generally, for membrane-swelling although it produces cytolysis, does not render the plasma-membrane readily permeable to salts. Indeed in hypertonic salt solutions, eggs with swollen membranes shrink even more than those with unswollen membranes (see pp. 155–158). This is sufficient proof that the swollen membrane retains its semipermeability.

In an earlier paper it was pointed out that all substances which had been described as producing membrane elevation (as well as some other newly discovered ones) did actually lower surface tension in aqueous solution. In a few substances, however, this lowering could apparently only be a very slight one. These cases have been reinvestigated, and they have been found to involve membrane swelling rather than membrane elevation.

When membrane swelling is rapid, it is possible that the lowered surface tension so produced should be followed by membrane elevation if the eggs are immediately returned to sea-water. One such case was indeed observed, but the observation was made at the close of the season, and further study of this point is necessary.

D. Cortical Action of Heat and of Various Chemicals.

Heat.—Although heat has been described as producing artificial parthenogenesis in the sea-urchin egg (McClendon, '10), I know of no description of membrane elevation as a result of heat treatment. In several experiments, I was unsuccessful in producing membrane elevation in this fashion. In experiment *a*, eggs were dropped into sea-water which had been heated to 36.5 degrees, and were exposed 1, 2, 3, 4 minutes. In experiment *b*, the eggs were dropped into sea-water heated to 35 degrees, and were exposed 2, 4, 6, 8 minutes. In experiment *c*, eggs were dropped into sea-water heated to 37.5 degrees and kept at this temperature on a waterbath; the eggs were exposed 3¼ minutes. In no case could any membrane elevation be observed, but the vitelline membrane did appear to be slightly swollen after the heat treatment.

Alkalis.—If alkali is added to sea-water, the resultant precipitation of magnesium salts tend to convert the sea-water into a solution containing little else than sodium salts. No doubt this fact alone is of significance. It is probable, however, that there is a more specific action of the alkali. As was shown in a previous discussion (p. 151), alkali apparently is able to liquefy the vitelline membrane. It is doubtful if any observer has actually obtained membrane elevation with NaOH or KOH. Certainly it does not occur when the eggs are left in the alkaline solution.

Butyric Acid.—This famous method is one of the best for artificial membrane elevation. Loeb used it originally on *Strongylocentrotus* eggs, employing a concentration of 50 c.c. sea-water plus 2.8 c.c. *N*/10 butyric acid and exposing the eggs 1½–3 minutes. In his recent book, he applies the method to *Arbacia* eggs reducing the concentration of acid slightly (50 c.c. sea-water plus 2.0 c.c. *n*/10 butyric), but retaining the same time of exposure. He says that the eggs do not form a conspicuous fertilization membrane, but only a "fine gelatinous layer which was not easily visible." This is evidently a membrane swelling and not an elevated membrane. For the benefit of workers in the field, I mention a slight modification in the method, by which true elevated membranes may be obtained for *Arbacia* eggs. Instead of 1½–3 minute exposures, a ½ minute exposure was found very effective, and over ninety per cent. of elevated membrane could regularly be obtained if the eggs were well washed and in good condition. The concentration used was the original one of Loeb's, 50 c.c. sea-water plus 2.8 c.c. *N*/10 butyric; slight variations are however immaterial. Although eggs exposed 1 minute often showed membrane elevation, no elevation was found to occur on longer exposure. Instead the membrane in every case appeared swollen, the amount of swelling increasing with the length of exposure.

Inorganic Salts.—That isotonic solutions of various salts cause membrane "formation" was discovered by R. Lillie ('10). The effectiveness of the various salts was found to correspond with their order in the lyotropic series, *i. e.*, those salts which are most effective in producing swelling of protein gels (in alkaline solution) were also most effective in inducing membrane "formation." The reason for this correspondence becomes apparent, when we consider that the membrane "formation" produced by isotonic salt solutions is nothing other than a membrane swelling. The membranes resulting from salt treatment are sticky, they do not collapse in the presence of a colloid (cf. p. 160).

The concentration of a salt is an important factor in determining whether swelling shall take place, and how pronounced the swelling shall be. Let us consider as an example the case of NaCl. Seven solutions of NaCl were prepared by adding distilled water to a 2.5 normal solution.

Thus Solution *A* contained	50 c.c. $2\frac{1}{2}$ *M* NaCl plus	0 c.c. distilled water.
B	40	10
C	30	20
D	20	30
E	10	40
F	5	45
G	$2\frac{1}{2}$	47.5

Eggs were placed in all these solutions at 3:30 P.M. By 3:39 eggs in *A* showed a membrane swollen to 2.5μ in diameter. By 4:50 it had reached approximately 5μ.

As for the eggs placed in *B*, the membrane swelled at least as rapidly as in the first case.

Membranes on eggs in *C* and *D* also swelled rapidly, but in no case did eggs in *E*, *F*, *G* show any membrane swelling. Thus a certain concentration is necessary for swelling to occur.

There appears to be at least two factors involved in this effect of concentration. In the first place, the swelling action of the salt no doubt increases with the concentration. But it is probable that the hypertonicity of the solution may also play a rôle.[1]

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[1] The explanation of such an effect might be as follows: When water is extracted from the cell, there is a tendency for vacuum production and consequent negative pressure (release of pressure) on the particles of the membrane. But we know

Sensitization with Strontium Chloride.—The process of sensitization was originally used by Loeb, later by Robertson, to induce the "formation" of a membrane in eggs exposed either to blood sera or to various tissue extracts, all dissoved in what was essentially a NaCl solution isotonic with sea-water. The method employed consists either of adding 3/8 *M* $SrCl_2$ directly to the serum, or of placing the eggs first into the 3/8 *M* $SrCl_2$ for several minutes, then into the serum. In the first paper of this series I urged that $SrCl_2$ caused a precipitation of sulphates and that much of the effect of sensitization was no doubt due to this action. In the summer of 1913, I was able to prove the truth of this statement. On July 31, 10 c.c. of 3/8 *M* $SrCl_2$ was added to 90 c.c. of sea-water. The voluminous precipitate of $SrSO_4$ was allowed to settle and filtered off, but the precipitate still continued to settle from the filtrate. When eggs were placed into this filtrate membrane swelling occurred, and this was followed by cytolysis. In another experiment, the eggs were placed first into 3/8 *M* $SrCl_2$ and then into a 0.55 *M* NaCl solution. Used alone, the NaCl solution did not cause membrane swelling when two drops of a thick egg suspension were placed into 75 c.c. of it. But when several drops of egg suspension were placed into 3/8 *M* $SrCl_2$, and then after five minutes, 2 drops of liquid containing eggs were taken from the $SrCl_2$ solution and placed into the NaCl solution, membrane swelling could be observed to take place in the eggs so transferred. The vitelline membrane could be seen slowly to increase in thickness, so that after about half an hour a good proportion of the eggs were surrounded by a transparent outer layer which bore a close resemblance to an elevated membrane, except for the fact that the inner boundary of such a membrane was absent. The membranes produced as a result of the "sensitization" process did not collapse in the presence of a colloid. They were also sticky, and as a result the eggs tended to agglutinate. Such an agglutination of eggs with swollen membranes has no doubt led Robertson ('12) to the view that "fertilization and agglutination are similar phenomena."

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from Le Chatelier's theorem that whenever the pressure on a system in equilibrium is diminished, a change or reaction ensues which is accompanied by increase of volume. Hence the swelling.

These facts enable us to interpret many of the results of Loeb and Robertson on a truly chemical basis, and without the aid of a hypothetical lysin. But there is also another factor to be considered in the analysis of the action of sera, and tissue extracts. It is the fact that many proteins are probably able to effect a swelling of the vitelline membrane. Such an action is at least true for egg albumen. The eggs of 2 females were washed twice and pipetted into 100 c.c. of 1 per cent. egg albumen in sea-water. Before ten minutes had elapsed the membranes on most but not all of the eggs had swollen considerably. The swelling action of albumen seems to vary considerably, sometimes being almost negligible. It is, I think, to be regarded as a phenomenon akin to peptonization, the albumen playing the part of a protective colloid.

KCN.—The action of potassium cyanide is rather difficult to explain on basis of the surface-tension theory. Although KCN alone, in the dilutions used, never caused elevation of the membrane, if the solution was made hypertonic the membrane did separate from the egg. Thus on July 31, 1914, membrane elevation did not occur in 25 c.c. of sea-water plus 1 c.c. of 1/20 per cent. KCN, but in a similar solution plus 4 c.c. 2.5 *M* NaCl, the vitelline membrane in practically every case became lifted away from the egg surface. How can this observation, which was repeated several times, be interpreted on the basis of the surface-tension theory? As is well known, KCN hydrolizes readily, so that KOH and HCN are always present in a solution of the cyanide. Probably HCN plays the most important part, for it is a gas, and hence its surface tension is practically zero. Thus it no doubt lowers the surface tension of the vitelline membrane (cf. p. 166). But membrane elevation does not result, because of another effect of the cyanide. In the first paper of this series, it was pointed out that even if a substance lowered surface tension, it would not produce membrane elevation if it increased too greatly the modulus of elasticity of the vitelline membrane, for in that case the toughened membrane would be incapable of stretching. There is evidence that KCN actually does increase the modulus of elasticity. In the next section it will be shown that the presence of KCN retards membrane swelling, and such anti-swelling action is a general characteristic of all substances which increase the modulus of elasticity.[1] As a result of the stiffening action of KCN, the membrane resists elevation, for the elevating force is not sufficient to stretch it. But in a hypertonic solution, as the egg shrinks, no stretching is necessary to separate the vitelline membrane from the egg, and the membrane is accordingly pulled away from the egg surface.

E. Inhibitors to Membrane Swelling.

It has been found that swelling of the vitelline membrane may be retarded or even inhibited in the presence of certain substances. Up to the present only two such inhibiting substances have been discovered. Of these, KCN inhibits the swelling of the membrane by salts, sea-urchin blood on the other hand inhibits acid swelling. But the inhibitor of salt action has no such effect on acid swelling, and sea-urchin blood instead of inhibiting swelling produced by salts, actually seems to favor it. These results, paradoxical as they appear at first sight, are really in direct line with recent findings in the field of colloid chemistry. There it has been shown that the salt and the acid swellings of gelatine must be essentially different processes, for the very salts which, by themselves, favor or even cause swelling, retard the swelling effect of an acid.

Although membrane swelling usually occurs in "NaCl hypertonic sea-water,"[2] in the presence of traces of KCN no such swelling will occur. This inhibiting effect of KCN has as yet not been found to be shared by any other substance, but so far only a few experiments in this direction have been made. Some of the experiments made during the summers of 1913 and 1914 are recorded below.

August 29, 1913. To 200 c.c. of sea-water was added 32 c.c. of 2.5 M NaCl and the resultant "NaCl hypertonic sea-water" was divided into four 50 c.c. portions, A, B, C, D. To A was added 0.5 c.c. 1 per cent. KCN, to B 0.5 c.c. $N/10$ NaOH, to C, 0.5 c.c. ether, and nothing was added to D, which several as a control.

Eggs were then placed in A, B, C, D, and it was found that although membrane swelling very evidently took place in D, no such swelling occurred in *A*. The effect was not due to the alkaline reaction of KCN, for the swelling of egg membranes in *B* showed that NaOH had no retarding effect on the process. Likewise the ether present in *C* appeared to have no inhibiting effect.

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[1] Freundlich, "Kapillarchemie," p. 512.

[2] As previously stated, I use this term to indicate a solution of 50 parts (by volume) of sea-water plus 8 parts of $2\frac{1}{2}$ M NaCl.

In other experiments KCN behaved similarly. In general it was found advisable to first place the eggs into sea-water which contained KCN and to add the 2.5 *M* NaCl later. Thus on August 29, 1914, it was found that some membrane swelling occurred when eggs were dropped into hypertonic sea-water to which KCN had already been added, but that this was entirely prevented when the eggs were first exposed to the KCN solution in sea-water, the 2.5 *M* NaCl being added later.

August 29, 1914. Fingerbowls *A*, *C*, *D*, were used. Into *A* were placed 50 c.c. sea-water plus 8 drops of egg suspension, and to this were added 8 c.c. of 2.5 *M* NaCl. Fingerbowl *C* contained 50 c.c. sea-water plus 1 c.c. 1/5 per cent. KCN, to this were added at 10:30½ A.M. 8 c.c. 2.5 *M* NaCl, and at 10:31 A.M. 8 drops of egg suspension were dropped into the cyanide containing "NaCl hypertonic sea-water." Fingerbowl *D* contained 49 c.c. of sea-water plus 1 c.c. 1/5 per cent. KCN. Several drops of egg suspension were added to this solution of cyanide in sea-water, at 10:40 A.M. At 11:28 A.M. (48 minutes later), 8 c.c. of 2.5 *M* NaCl were added to *D*.

On microscopical examination, it was found that pronounced membrane swelling had taken place in *A* (in the absence of KCN), swelling also occurred in *C*, but did not appear to be pronounced as that occurring in *A*. No membrane swelling at all could be observed in *D*, in which the eggs had been treated first with KCN before the concentrated NaCl solution had been added.

The above experiment indicates that the action of KCN in inhibiting membrane swelling produced by NaCl, is not the result of a reaction between the cyanide and the salt, but is due to an effect of the cyanide on the membrane. For if only a reaction between salt and cyanide was involved, there could be no advantage in first subjecting the eggs to the action of the cyanide alone.

Although KCN inhibits the membrane swelling effect of

NaCl, it does not appear to have the slightest retarding effect on membrane swelling when this is produced by an acid.

August 28, 1914. Fingerbowl *A* contained 50 c.c. sea-water plus 3 c.c. *N*/10 butyric acid. Fingerbowl *B* contained 49 c.c. sea-water plus 3 c.c. *N*/10 butyric acid plus 1 c.c. 1/10 per cent. KCN. When eggs were added to *A* and *B*, membrane swelling occurred in both.

September 3, 1914. Stender dish *A* contained some eggs in 25 c.c. of sea-water. To this was added 1 c.c. 1/5 per cent. KCN at 10:52 A.M. Then 2 c.c. *N*/10 butyric acid were added 29 minutes later (at 11:21).

Stender dish *B* contained eggs in 25 c.c. of sea-water, 2 c.c. of *N*/10 butyric acid were added at 11:22 A.M.

In both *A* and *B*, membrane swelling occurred and as a result the eggs in both cases stuck to each other and to the bottom of the dish. No difference could be observed between the two sets of eggs, and apparently the KCN has no effect on acid swelling of the membrane.

KCN is thus capable of inhibiting membrane swelling by NaCl, but it has apparently no effect when the swelling is produced by butyric acid. On the other hand, sea-urchin blood was found to retard or inhibit acid swelling.

June 22, 1914. A solution of butyric acid in sea-water was prepared by adding to 50 c.c. of sea-water, 2.5 c.c. of *N*/10 butyric acid. Approximately 5 c.c. of the resulting solution were placed in each of two Syracuse watch-crystals (*A* and *B*). To watch-crystal *A* were added 3 c.c. of filtered sea-urchin blood. (The blood was filtered after it had been allowed to "clot" by standing.) To watch-crystal *B*, 3 c.c. of sea-water were added. In *B*, membrane swelling and agglutination occurred, in *A* very little, if any, membrane swelling occurred, and there was no agglutination. The jelly was dissolved away from the eggs in *A*, but although the eggs were thus able to come into close contact, they would separate again, showing that they were not sticky, and that no membrane swelling had occurred. After a few hours, eggs in *B* had completely lost their color and appeared white to the naked eye, those in *A* appeared normal.

July 7, 1914. The above experiment was repeated. In this case an acid solution was made up by adding 5 c.c. *N*/10 butyric acid to 50 c.c. of sea-water. Of this solution, 5 c.c. was put into each of two Syracuse watch-crystals *A* and *B*.

To *A* were added 5 c.c. of sea-water and five drops of egg suspension.

To *B* were added 5 c.c. of filtered blood (from several ♂s and ♀s) and then 5 drops of egg suspension.

The result of the experiment was that in *A* the vitelline membranes swelled in practically every case. A count of a hundred eggs gave ninety-nine eggs with swollen membranes and the single exception was doubtful. On the contrary, there was practically no membrane swelling in *B*, which contained blood in addition to the acid. A count gave, of a hundred eggs observed, only three with swollen membranes.

The inhibiting effect of blood upon acid swelling, unlike the effect of cyanide on salt swelling, may perhaps be the result of a direct action of the blood upon the acid. This is barely suggested by the fact that *N*/10 HCl produces a flocculent white precipitate when added to filtered sea-urchin blood. However in the above recorded experiments with butyric acid, no such precipitation could be observed.

Instead of exhibiting a retarding effect upon membrane swelling by salts, sea-urchin blood seemed to favor the process. This favorable effect was much more pronounced in some cases than in others, and in several experiments it was not readily observed. On July 28, 1914, membrane swelling was found to be much more rapid and pronounced in a solution of 20 c.c. sea-water plus 5 c.c. filtered blood (from ♀s) plus 4 c.c. 2.5 *M* NaCl, than in a similar solution without blood, *i. e.*, 25 c.c. sea-water plus 4 c.c. 2.5 *M* NaCl. It sometimes happens, and this appeared to be more frequent in 1914 than in 1913, that membrane swelling does not occur in "NaCl hypertonic sea-water." In such cases, it was found possible in several instances to produce a membrane swelling by the addition of blood. Thus on July 30, 1914, although there was no swelling in "NaCl hypertonic sea-water," when eggs of the same lot were placed into 5 c.c. blood plus 20 c.c. sea-water plus 4 c.c. 2.5 *M* NaCl membrane swelling did occur.

It might be reasoned that this action of blood is analogous to the cytolytic effect of sera foreign to the individual. But in one case at least, blood of the same individual from which the eggs had been taken, was found to exert an accelerating effect upon membrane swelling in "NaCl hypertonic sea-water."

If it be true that blood retards acid swelling and favors salt swelling, this fact can be used in cases of doubt, to determine if a given type of membrane swelling is the result of the action of a salt or an acid.

F. Cortical Changes at Fertilization.

The central object of studies in artificial parthenogensis is to find an explanation of the processes occurring in normal fertilization. The fact that artificial membrane elevation is apparently always the result of a lowered tension of the vitelline membrane has of course led to the view that the spermatozoön also produces a lowered surface tension. There are two conceivable ways in which this could happen. In the first place the sperm might carry a substance which lowers surface tension directly. This is improbable, in view of the fact that it has not been possible to extract from sperm a membrane-elevating substance. It is more logical to suppose that the very act of penetrating on the part of the sperm lowers the tension.

If the tension of a stretched thread be lowered at one point, instantaneously the tension throughout the thread is lowered. Similarly if the tension of a spherical stretched film or membrane be lowered at one point, there will be a lowering of tension in every point of the film, for in order that equilibrium be established, the tension in every part of the spherical film must be equal. This equalization of tension is probably a rapid process, especially when not merely a point, but an appreciable area of the surface has its tension lowered.[1]

Thus the penetration of the sperm almost immediately produces a lowered tension in all parts of the vitelline membrane. In the sea-urchin egg, the sperm can not bore its way through the membrane mechanically, as it is not provided with a perferatorium. It is therefore probable that the sperm has a solvent action on the membrane upon coming in contact with it. The partial liquefaction or swelling of the vitelline membrane at the point of sperm entrance can be conceived of as serving two functions: (1) it enables the sperm to enter, (2) it lowers the surface tension of the membrane and thus produces membrane elevation.[1]

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[1] However, in cases where a very thin film surrounds a large spherical mass as in air-bubbles the attainment of equilibrium between the parts of the film is much slower than the attainment of equilibrium between the film and the contiguous fluids. *Cf.* Gibbs, *l. c.*, p. 300.

That the sperm actually does produce a substance capable of causing membrane swelling, can be demonstrated. It is not possible to observe the swelling produced by a single sperm. But if the eggs are placed into very concentrated sperm suspensions, the vitelline membrane can be seen to swell all around the egg. Such concentrated suspensions are obtained by allowing the sea-urchins to shed their sperm. As is well known, the shedding reaction is aroused when the oral part of the shell is cut away. If the "dry" sperm be diluted only very slightly, an enormous sperm concentration can be obtained. When eggs are mixed with sperm suspensions of such high concentration, each egg immediately becomes surrounded by a halo of wriggling sperm. Soon the vitelline membrane can be seen slowly to increase in thickness, it swells until it may reach a thickness of about 3 microns. That normal membrane elevation has not taken place can be shown by the fact that the swollen membranes thus produced do not collapse when the eggs are placed in a 1 per cent. or a 2 per cent. albumen solution (in sea-water). In a concentrated sperm suspension, each point on the vitelline membrane is a point of attack on the part of the spermatozoa, and the entire membrane becomes swollen.[2]

The concentration of sperm necessary to produce a complete swelling of the membrane varies with the season. In the height of the season only quite concentrated sperm suspensions produce the phenomenon. But towards the end of August, as the season begins to wane, more and more dilute suspensions become effective, until at the very close of the season, it is difficult to secure true membrane elevation at all. Probably the fluid emitted with the sperm is then charged with the substance which causes the swelling.

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[1] It might be thought that puncture of the vitelline membrane, *e. g.*, by a needle, should produce elevation. But this is not necessarily the case, for the hole produced by a mechanical puncture of the membrane, if not immediately closed, would involve a loss of its semipermeable properties, and these on the basis of the theory (see pp. 165–166) are necessary for membrane elevation. A deep prick would also produce coagulation of the underlying cytoplasm, which would tend to prevent elevation.

[2] It might be asked why elevation of the membrane does not follow swelling produced by concentrated sperm suspensions, since this no doubt results in a rapid lowering of surface tension. The answer is clear. In a previous paper (Heilbrunn, '13) it was pointed out that membrane elevation never occurred when the egg or its cortex was coagulated. Now in concentrated sperm suspensions, it can be shown that a profound coagulation does take place. This was demonstrated by a method which has been developed for revealing the presence of coagulation within the egg. For details of this method see p. 192.

In the preceding section it was shown that sea-urchin blood retards acid membrane swelling, but favors salt membrane swelling. This fact makes it possible to determine the general nature of the membrane swelling produced by sperm. One has only to observe the action of concentrated sperm suspensions in the presence of blood. If blood favors the swelling, this can be taken as evidence that the sperm action is similar to that of salts, if on the other hand it retards swelling, an acid is probably responsible. On July 7, 1914, 5 c.c. of sea-water plus 9 drops of egg suspension plus 5 drops of "dry" sperm were mixed in Syracuse watch-crystal *A*. Watch-crystal *B* contained 4 c.c. of blood (filtered from ♂s and ♀s) plus 9 drops of egg suspension plus approximately the same amount of sperm as did *A*. Both watch-crystals were shaken slightly to insure mixing. In *A*, the membranes swelled gradually; after 43 minutes they measured approximately 2μ. In *B*, on the contrary, no membrane swelling could be observed.

It might be objected that in the above experiment the blood had some effect which prevented intimate contact of sperm with egg. This objection is obviated by the following experiment. It was performed at the very close of the season, at a time when as previously pointed out, sperm suspensions have a much greater tendency to produce swelling. On September 1, 1914, considerable difficulty was experienced in procuring sperm. Ten males were cut open and allowed to shed (several others had been rejected as being totally incapable). Of these ten, only two shed any sperm at all, and this was rather watery. Preliminary experiments showed that when 3 drops of this watery "dry" sperm were added to about 10 c.c. of sea-water and about 8 drops of the resulting suspension were again diluted with 10 c.c. of sea-water, a suspension was obtained which caused membrane swelling when 5 drops were added to 25 c.c. of sea-water, but only a moderate per cent. of fertilization when 1 drop was added to 25 c.c. of sea-water containing eggs. In the main experiment 3 drops of watery "dry" sperm were added to approximately 10 c.c. of sea-water, and the resulting suspension was the one used. Two Syracuse watch-crystals were employed. Watch-crystal *A* contained 4 c.c. of sea-water plus 1 c.c. of filtered blood (from ♀s). Watch-crystal *B* contained 5 c.c. of sea-water and no blood. 8 drops of a dilute egg suspension were then added to *A* and to *B* (at 11:15½ A.M.) and two minutes later, 2 drops of the sterm suspension just mentioned, were added. At 11:20, eggs in *B* (*i. e.*, in the absence of blood) all had membranes widely swollen all around. At 11:22, the eggs in *A* were examined. Most of the eggs showed not a trace of cortical change, but some showed membrane *elevation*. Shortly after, a count was made of eggs in *A*. It was found that 23 showed no cortical change, 1 was doubtful, it may have had a swollen membrane, 9 evidently possessed elevated membranes.

Thus the presence of blood prevents membrane swelling, and as one result of this prevention of excessive swelling, membrane elevation is possible, although without blood it could not have been produced. In this experiment, the anti-swelling effect of blood towards sperm suspension is conclusively demonstrated, for the blood evidently does not prevent access of sperm.[1]

The fact that the presence of blood inhibits membrane swelling in concentrated sperm suspensions, indicates that the swelling is produced by an acid. As is well known, all spermatozoa are abundantly provided with nucleic acid, and it is very probable that a nucleic acid or its derivative is responsible for the swelling.

I have tried a number of times to watch the process of normal membrane elevation under high power, but with only scant success. The presence of a coverslip produces difficulties. If a drop of egg suspension is placed on each of two slides, and one drop is then covered by a coverslip with a drop of sperm suspension on its lower surface, and to the other drop is added a drop of the same sperm suspension, but no coverslip, a marked difference between the two preparations can be noted. In the absence of a coverslip, a much greater per cent. of eggs undergo membrane elevation. When the drop of sperm suspension is added to the eggs after the coverslip has been placed in position, practically no membrane elevation occurs. In these experiments, the coverslip was always supported by strips of paper or thin glass tubes, so that there could be no question of compressing the eggs.

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[1] The further history of the eggs in this experiment is very interesting. On September 2, there are considerably fewer larvæ in *A* (where blood was present) than in *B*. But whereas all of the larvæ in *A* have well-marked arms, none of the larvæ in *B* possess even the suggestion of arms. On September 3, the larvæ in *B* are still perfectly armless, whereas those in *A* have the usual long arms of the pluteus stage.

The effect of the coverslip is in part due to the action of the glass (or of substances diffusing out of it)[1] on the spermatozoa. The sperm apparently congregate at the surface of the coverslip. But this is not believed to be the only effect, and some evidence that I possess, although not absolutely unimpeachable, tends to show that the pressure of the coverslip is also partly responsible for preventing elevation. However, further experiments on this point are necessary; I merely bring up the matter here in order to emphasize the difficulties in the way of direct observation. Fol ('79) speaks of the great difficulty in observing fertilization in the sea-urchin egg. Pictet ('91) found no such difficulty, but I think that the cortical effect that he describes was not membrane elevation, but membrane swelling, which is not retarded by the presence of the coverslip.[2]

My observations, though admittedly incomplete, tend to show that the membrane is elevated from all sides of the egg at the same moment. It is possible that elevation does start at the point of sperm entrance as Ries ('09) for example claims, but if this part of the membrane does show any priority, it is only an exceedingly brief one.

On the basis of Loeb's view that the sperm contains a lysin which produces membrane "formation" directly, we would have to suppose that this lysin diffuses around the egg surface in incredibly fast time. The morphology of sperm membrane elevation offers a severe obstacle to most theories which attempt to account for the process, but it is readily understandable on the basis of the surface tension theory. The membrane swelling which occurs at the point of sperm entrance, causes a lowering of surface tension not only in the immediate vicinity, but everywhere on the egg surface. Hence the vitelline membrane is lifted from all parts of the egg at practically the same moment.

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[1] Possibly dissolved out by the alkali of sea-water.

[2] Both Pictet and Fol subjected the eggs to slight compression.

G. The Significance of Cortical Change.

Although it has never been proven that cortical change is absolutely essential before development can take place, it is generally admitted that membrane "formation" must precede any normal development. We have seen that cortical change or membrane "formation" may involve either of two quite different processes. What then is the fundamental significance of cortical change, why is it necessary for normal development? I think that at least part of the answer is forthcoming. If an egg be watched under a micrometer scale, it will be noticed that shortly before the first cleavage the egg rapidly lengthens in the direction of its polar axes. In three minutes, an egg was in one case observed to increase its polar axis by 6.5μ, in another case by 8μ. (This fact is by no means new, all the pictures of cleavage show an increase in polar axis.) If the egg is surrounded by a stiff membrane, such a rapid change of form could scarcely be possible. But cortical change, whether it be membrane swelling or elevation, always results in the removal of this obstacle. The vitelline membrane is either rendered soft by swelling, or it is lifted away from the egg surface and its place taken by the no doubt less rigid hyaline layer. As a result, rapid changes in egg form can occur. Moreover, as is well known, the hyaline layer is normally pulled in between the first two blastomeres during the cleavage process. The stiff vitelline membrane could scarcely act in this way. But either membrane swelling or membrane elevation would result in the egg being invested by a membrane which was not too rigid to be pulled in. Thus at least two processes which play a part in normal development would be greatly hindered if some kind of cortical change did not occur.

Moreover when hypertonic solutions are used as reagents, the degree of rigidity of the plasma membrane becomes a factor of importance. As was shown previously (p. 158), exosmosis is favored by a cortical change which tends to soften the vitelline membrane.

III. Internal Change.—The Problem of Segmentation

A. Theories of Segmentation.

The real problem in the study of artificial parthenogenesis is not the problem of cortical change, but is rather the analysis of the factors which produce initiation of development. How can we define initiation of development? Although it has been shown that after fertilization there is a quickening of various energetic processes in the sea-urchin egg, plausible as such a view no doubt is, no one has ever shown that all the recorded instances of artificial parthenogenesis involve an increased metabolism. In practically every case, segmentation or mitosis has been regarded as the sole criterion of initiation of development. This is, I believe, wholly justifiable, as the act of segmentation is in itself a beginning of development.

Of the various theories of artificial parthenogenesis, those of R. Lillie and of Loeb have in recent years met with the most favor. The former believes that the initial change is an increase in permeability of the plasma membrane. This is the direct cause of (1) mitosis, and (2) increased metabolism. In his theory of mitosis (R. Lillie '11), now widely accepted as the least objectionable of any of the numerous theories of mitosis, he considers the hypothetical plasma membrane as charged by an electrical double layer; this charge is neutralized upon an increase in permeability. Such a neutralization of charge produces, according to Lillie, a lower potential at the surface as compared with the interior, and the difference in potential thus produced brings about a number of internal changes, which lead finally to the formation of a mitotic spindle. I confess that I am unable to understand the fundamental basis of the theory. It appears to me that any drop in the potential at the surface of a body involves *simultaneously* an exactly equivalent drop of the potential at every point in the interior. This seems a direct consequence of the textbook definition of potential at a point, as the amount of work done by the electric field on unit charge, when it is brought to that point from infinity.

Moreover, even if we could accept some of the extremely dubious evidence that has been offered in favor of increased permeability, no one has ever tried to show that all parthenogenetic agents do cause increased permeability. In fact R. Lillie has at times assumed that hypertonic sea-water and that magnesium salts cause a decrease rather than an increase in permeability, and yet hypertonic sea-water, even in the presence of magnesium salts, produces initiation of development.

Of Loeb's ideas concerning the initiation of development only a bare outline is possible here. Recently, he is "inclined to believe that in all cases in which an unfertilized egg has been caused to develop a typical or atypical membrane had been formed."[1] This membrane "formation," which, as we have seen, Loeb regards as a swelling process, is the important initiative factor. It causes directly an increase of oxidations, but leaves the egg in a sickly condition, hence it is necessary to provide a corrective agent which may be either oxygen-containing hypertonic sea-water or the absence of oxygen. The theory of the necessity of the corrective factor need not concern us here, for we are primarily interested, not in the best method of obtaining artificial parthenogenesis, but rather in the nature of the physical or chemical change which causes *any* initiation of development. Loeb believes this change to be an increase in oxidations. In support of this view he has accumulated a mass of data. He first showed that in his original method of using a hypertonic solution alone, artificial parthenogenesis could only be produced in the presence of oxygen. More recently he has measured egg oxidations and has confirmed Warburg's observation that membrane "formation" produces a great increase in oxidations. According to Loeb's measurements, any cytolytic change results in a great increase of egg oxidations.

B. The Action of Hypertonic Sea-water in the Presence of KCN.

Loeb was originally led to adopt the oxidation theory, by the fact that either the addition of KCN, or the removal of oxygen by the passage of hydrogen, seemed to prevent the action of hypertonic solutions upon sea-urchin eggs. He reasoned from this that the hypertonic solutions produced an increase in oxidations. In 1913 and 1914, I performed a number of experiments with hypertonic solutions in the presence of KCN, or in an atmosphere of hydrogen. In this paper I shall, however, report only on the KCN experiments. The results obtained in an atmosphere of hydrogen are perhaps more interesting, but I do not feel as yet that every possible source of error has been eliminated.

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[1] Loeb, '13*a*, p. 223.

At present it is generally accepted by most physiologists that KCN suppresses cell oxidations.[1] Loeb states that 2 c.c. 1/20 per cent. KCN to 50 c.c. of sea-water suffice for the purpose.[2] In my experiments, a much higher concentration of the reagent was employed.

On August 19, 1913, I added 0.5 c.c. of 1 per cent. KCN to every 58 c.c. of "NaCl hypertonic sea-water." (This addition of only 0.5 c.c. of more concentrated reagent had the advantage of not incurring as much dilution as the addition of 2 c.c. of almost pure solvent.) Eggs were exposed to this cyanide-containing "NaCl hypertonic sea-water," as well as to the normal "NaCl" hypertonic sea-water. The results appear in the folfowing table, in which the numerators of the fractions indicate the number of eggs observed to segment, the denominators the total number of eggs counted. Before return to sea-water, the eggs which had been exposed to KCN were washed twice by Lyon's test-tube method (Lyon '02), so that all trace of the poison might be removed.

Minutes Exposed.	"NaCl Hypertonic Sea-water."	"NaCl Hypertonic Sea-water" plus KCN.
25	127/209	10/102
30	83/100	9/108
35	62/100	22/247

Of the eggs exposed 25 or 30 minutes to the cyanide-containing hypertonic solution, none went beyond the 2 (or 3)-celled stage, but of the 22 eggs which segmented after an exposure of 35 minutes, two became many-celled blastulæ and may have gone

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[1] Personally I do not believe that the evidence in support of this view is conclusive.

[2] Loeb, '09, p. 55.

farther, one reached a few-celled blastula stage. The control of unfertilized eggs in sea-water showed practically no segmentation, a count showed three out of more than five thousand.

In the above experiment only about 30–40 per cent. of the eggs underwent membrane elevation. In other experiments, in which less concentrated solutions of KCN were employed, all cortical change was inhibited and the eggs did not segment, although nuclear division sometimes occurred. In order to obtain the best results, widely elevated membranes must be obtained. If the membranes were only lifted a short distance from the egg in the cyanide-containing hypertonic sea-water, on a return to normal sea-water the egg expanded until it again came into contact with the membrane (often it ruptured the membrane and an exovate was produced). The best results were obtained in an experiment of September 2, 1914. In this case, 1 c.c. of 1/5 per cent. KCN was added to 24.5 c.c. of sea-water containing eggs, at 11:45 A.M. At 11:45 1/6 A. M., 4 c.c. of 2.5 *M* NaCl were added, so that the eggs were then in "NaCl hypertonic sea-water" plus KCN. They were allowed to remain in this solution for 33 minutes, during which time it was noticed that ninety-seven per cent. underwent membrane elevation. At 12:18 P.M., some eggs in 4 c.c. of the solution were transferred to about 600 c.c. of sea-water in a tall graduate. At about 12:45 P. M., most of the sea-water was siphoned off from the graduate and fresh sea-water added. At 2:30 P.M., out of 233 eggs examined 22 were found to have segmented, but the count was evidently too low, as doubtful cases were rejected. In some instances, exovates simulated cleavage. Only those eggs in which a nucleus could be observed in each cell were counted. At 4:55 P.M., 50 eggs, out of 110 examined, were found to have segmented, but this count was likewise probably too low. At 9 P.M., hundreds of motile blastulæ could be observed. (Of 337 eggs noted, 28 were motile larvæ, of these 18 swam on the bottom, 10 on the top.)

These experiments indicate that the action of the hypertonic solutions in initiating development is not due to an effect on the oxidative processes, for the hypertonic solution has the same action in the presence of a concentration of KCN, which according to Loeb is considerably above that sufficient to check oxidations.

Loeb also found that the presence of KCN prevented degenerative change in eggs exposed to hypertonic sea-water, and assumed that the KCN acted by retarding excessive oxidations. However on the basis of our knowledge concerning the anti-swelling effect of KCN (*cf.* p. 174), it is simpler to assume that the inhibition of membrane swelling is the prime cause in preventing disintegration by the hypertonic solution.

The main support of the oxidation theory no doubt lies in the actual measurements of oxidations made by Warburg and Loeb. The method by which these measurements were made has been criticized in a note in *Science* (Heilbrunn '15).

C. An Analysis of the Methods of Producing Segmentation in the Unfertilized Arbacia Egg.

Hitherto in the study of artificial parthenogenesis, the general tendency has been to find new and different methods, rather than to find points of resemblance in the various means employed to produce segmentation in any one egg. The result has been that too much emphasis has been placed on the diversity and the unrelated character of the numerous parthenogenetic agents.

As a matter of fact there are in general only two ways of producing segmentation in the *Arbacia* egg, the endosmotic method and the exosmotic method. Whenever a reagent lowers the surface tension of the plasma-membrane, endosmosis is the result. The theoretical reason for this has already been considered (*cf.* p. 168). All those reagents which induce either true membrane elevation or membrane swelling are thus included in this category. In the first case the reagent produces a lowered surface-tension directly, in the second case, the swelling of the membrane results in a lowering of tension. Practically all of the reagents which have been used in artificial parthenogenesis produce either the one type of cortical change or the other. As a result, endosmosis follows, unless the eggs are in a hypertonic solution. In the latter case, water is extracted from the cell, and exosmosis occurs.

An apparent exception is found in the action of cold, which has been stated to produce parthenogenesis in the sea-urchin egg (McClendon '10). Lowered temperatures should result in an increase, rather than a decrease of surface tension, nor can they be thought of as causing exosmosis.

In the only experiment tried, I found that instead of producing artificial parthenogenesis, lowered temperatures tended to retard the natural parthenogenesis which is usually manifested by the Arbacia egg. On June 25, the temperature of the aquarium in which the sea-urchins were kept, was 19.5°, the temperature of the sea-water as it emerged from the tap was 19°; the room temperature (at 4.45 P.M.) was 24.5°. At 10.40 A.M., a beaker containing eggs in a small amount of sea-water was placed in a large beaker, and the space intervening between the two beakers was filled with cracked ice. The beakers were then placed in the ice-box. At 11.00 A.M., the temperature of the sea-water surrounding the eggs was 3.5°, at 11.35 A.M. it was 1.5°, at 2.15 P.M. it was 0.5°, and at 4.15 P.M. it was 1.5°. Eggs were transferred from cold to normal sea-water after 3½ hours (2.15 P.M.), 5 hours (3.40 P.M.), and 6½ hours (5.05 P.M.). We can refer to these three lots of eggs as lot *A*, lot *B*, lot *C*, respectively.

Lot *A* when counted at 4.30 P.M. showed one doubtful case of segmentation, out of 100 eggs observed.

Lot *B* at 5.30 P.M., out of 1,000 eggs counted, showed 3 eggs apparently cleaving irregularly, and 1 doubtful case. At best 4/1000.

Lot *C* at 8.55 P.M. showed 9/1000 cleavages.

The control of untreated eggs (from the same female) at 4.40 P.M. showed, of 1,100 eggs counted, 4 eggs cleaving irregularly (1 a 7-celled stage) and 5 with attempted or incomplete cleavages. At 9.05 P.M. however, the control showed a much higher count. Of 526 eggs counted, 36 showed irregular divisions. Thus of the eggs counted at about 9.00 o'clock, those which had been exposed to cold for 6½ hours showed less than 1 per cent. of segmenting eggs, whereas the control showed over 6 per cent. Thus the cold evidently retarded the process of natural parthenogenesis.

Of the two general methods of obtaining parthenogenesis, the exosmotic method yields the better results. Usually the endosmotic method produces only a small per cent. of segmenting eggs. But this is probably due to the specific poisoning action of the substances used in lowering surface tension, for when the sea-water is simply diluted, or when a harmless substance like egg albumen is used, much higher per cents. of dividing eggs are obtained. For example, when eggs were subjected to the action of a 1 per cent. solution of egg albumen (Kahlbaum) in sea-water, more than half of them divided, as shown in the accompanying table. In such a solution, the eggs were observed to undergo membrane swelling (see p. 173) and this was followed by endosmosis as shown by the increased diameter.

Length of Exposure to 1 Per Cent. Egg Albumen.	Segmentations.
30 minutes	55/100
66	48/100
118	50/100
233	6/100

The results gained with the endosmotic method used alone, are never as good as those which can be obtained with the exosmotic method. Neither are the per cents. of segmentation as high, nor is the degree of development attained as great. In spite of the fact that Loeb ascribes to hypertonic solutions a mere correcting effect, it is I think a noteworthy fact that no method of artificial parthenogenesis yet tried on the *Arbacia* egg, is truly effective, unless at some stage of the process it requires a hypertonic solution. In connection with this point, I made a number of experiments to test the butyric acid-KCN method which Loeb found so effective for *Stronglocentrotus*. I was at the time convinced that the method was essentially the same as Delage's acid and alkali method (Delage '07), and I tried to find if NaOH could not be substituted for the KCN, in other words if the correcting action of the latter was not due solely to its alkalinity. In no case, however, was I able to get any results either with KCN or NaOH as a "correcting agent" after butyric acid treatment. Indeed recently Loeb ('13*b*) has pointed out that the method is not suited for the *Arbacia* egg.

The question now arises if the two methods of producing segmentation, the one endosmotic, the other exosmotic, have anything in common. As a result of my experiments, I find that both methods produce a gelatinization or coagulation within the egg.[1]

Most biologists believe that the mitotic spindle is a condensation or coagulation product, and there is excellent support for this view. Hence any initiation of development must soon lead to some coagulation before mitosis can be accomplished. At present all students of artificial parthenogenesis, if they consider this coagulation at all, regard it as a secondary result. Thus, some think an increase of oxidations is first produced by all parthenogenetic agents, and that the increased oxidation involves changes which produce coagulation. Others think of the prime cause as an increase of permeability. But it is also possible to believe that the primary effect of all the parthenogenetic reagents is a coagulation effect. This view has had as its adherents, at one time or another, some of the foremost students of artificial parthenogenesis. In his earliest papers on the subject, Loeb occasionally leaned toward coagulation as a possible primary effect, but he soon abandoned the idea to become its vigorous opponent. Delage for many years maintained that artificial parthenogenesis is the result of a coagulation followed by a liquefaction, he considered membrane elevation as one evidence of such a coagulation. At present, however, (Delage and Goldschmidt '13), he favors the Lillie theory of increased permeability as affording a more probable explanation of the facts. Possibly the most vigorous and scientific attempt to support the coagulation theory was that of Fischer and Ostwald ('05). These workers argued from a theoretical standpoint that all parthenogenetic agents are of such a nature as to produce coagulation. Later Ostwald ('07) retreated from this view and admitted that coagulation might be only secondarily produced as a result of increased oxidation. That all parthenogenetic agents cause coagulation, was denied by Loeb.[1] He pointed out that benzol, toluol, and saponin are not protein coagulants. Since then the theory of Fischer and Ostwald had had no one to defend it.

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[1] In the test-tube, gelatinization and coagulation of proteins are apparently quite different phenomena, the former converting the entire mass into a jelly, the latter resulting in a separation of a precipitate. But in the small field of action of a sea-urchin egg, it would not be so easy to distinguish between the two, for the entire egg is smaller than a single flake of the usual precipitate. Moreover, even in the test-tube coagulation is usually preceded by a stage strictly comparable to gelatinization; the entire mass becomes opalescent and assumes a greatly increased viscosity; only later does the precipitate appear.

The view that I would maintain is that the only physicochemical effect which all parthenogenetic agents possess in common is the production of a gelatinization or coagulation within the egg. Hence I regard this gelatinization (or coagulation) as the direct cause of the initiation of development.

Leaving theory aside, it is possible to demonstrate that all parthenogenetic agents actually do produce gelatinization or coagulation within the egg. The distinguishing feature of a gel as opposed to a sol is its greater viscosity. All other macroscopic differences depend upon this. Indeed, according to Freundlich, the viscosity of a colloidal solution may be taken as a measure of its tendency to gelatinize ("Gelatinierungsbestreben").[1] The viscosity of the *Arbacia* egg protoplasm was regarded as an index of the state of aggregation of its constituents.

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[1] Loeb, '09, p. 217.

There are two general methods of measuring viscosity. One can either measure the rate of flow of a fluid, or one can study the movement of particles through the fluid. Both of these methods were used in studying the viscosity of the *Arbacia* cytoplasm. It is very easy to observe the rate of flow of the egg cytoplasm. One has only to exert pressure on the coverslip and the vitelline membrane soon bursts, allowing the egg contents to flow out. With this method, only great changes in viscosity can be noted, but gelatinization always does produce a very great increase in viscosity, so that the method usually suffices. The pressure can be applied by pushing on the coverslip with the point of a dissecting needle, or if greater accuracy is desired, a square piece of glass (broken from a slide) can be dropped from a known distance. This method of observing changes in viscosity is not entirely above criticism; for cortical changes, produced by the reagents used, might have an effect on the size of the aperture through which the cytoplasm flows.

The second method is much more exact and reliable. It involves a study of the movement of granules through the cytoplasm. The force which must be exerted to push the granules through the cytoplasm is a measure of the viscosity of the latter. Centrifugal force is of necessity used, and a hand centrifuge does very nicely.[2] Into one tube of the centrifuge are placed eggs which have been treated in various ways; into the other, normal eggs. After the centrifuging has been accomplished, a microscopic examination reveals any differences which may exist between normal and treated eggs. When normal unfertilized *Arbacia* eggs are centrifuged vigorously, the protoplasmic materials rapidly become separated into four layers or zones. Lyon ('07) has given an excellent description of the appearance of these zones and the reader is referred to his paper for details. The pigment-bearing granules come to be all massed at one pole, in the pigment zone; next to this is a zone of granular material, then a hyaline zone, and at the pole opposite the pigment zone is a small dense accumulation of substance which because of its color is known as the gray cap. The egg nucleus lies in the hyaline zone, directly beneath the gray cap. When an egg shows all these zones, I shall refer to it as "stratified." As the viscosity of the protoplasm increases, stratification becomes more and more difficult; in a thoroughly coagulated egg no stratification is possible.

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[1] Freundlich, '09, p. 416. Freundlich and Ishizaka, '13.

[2] A Bausch and Lomb instrument was used in my experiments, and the eggs were placed into the small glass tubes of the hæmatocrit attachment. New tubes were used in each experiment so that all danger of contamination was avoided. One turn of the high-speed handle involved 130 revolutions of the tubes. The distance between the end of each tube and the axis was approximately $7\frac{1}{2}$ cm.

Hypertonic solutions produce a very noticeable coagulative change in the sea-urchin egg. In 1913 a few preliminary experiments were performed in which the eggs were pressed out of shape by pushing down on the coverslip with a dissecting needle. It was found that "NaCl hypertonic sea-water" produced a marked increase in the viscosity of the cytoplasm. Such a solution causes swelling of the vitelline membrane. This swelling is apparently absent if a freshly prepared 0.49 M $MgCl_2$ solution is used. Eggs treated with this solution became very much more viscous than they had been previously. Whereas the normal unfertilized eggs shot out their contents rapidly if subjected to a slight pressure, eggs which had been immersed in $MgCl_2$ for 85 minutes could be subjected to considerable pressure without losing their circular outline. They could indeed be flattened out into a thin "pancake."

More accurate data were obtained with the centrifuging method. On July 23, 1914, some eggs were placed into 50 c.c. of sea-water plus 8 c.c. of 2.5 M NaCl at 2:21¾ P.M. At 2:44 P.M., these eggs were placed into one tube of the centrifuge, and into the other tube were placed some normal untreated eggs of the same female. At 2:45 P.M., after a few preliminary turns, the tubes were revolved for 28 seconds at a rate of 162 revolutions per second. The eggs were then examined. The normal eggs showed the typical zones of centrifuged eggs. The pigment zone extended for about one fourth of the egg diameter along the axis of stratification. The contrast between normal eggs and those which had been treated with the hypertonic solution was very marked. The latter as a rule showed no stratification whatsoever, although some eggs showed just the beginnings of such a process, the pigment granules being slightly more abundant at one pole of the egg than at the pole opposite to it.

The experiment was repeated on July 24. In this case unfertilized eggs were subjected to "NaCl hypertonic sea-water" at 10:29 A.M., and about 25 minutes later they were placed into one tube of the centrifuge. Into the other tube, normal eggs were placed. Then (at 10:55½ A.M.) the tubes were revolved for 19 seconds at the rate of 171 revolutions per second. Upon examination, the normal eggs showed complete stratification under low power of the microscope. High power examination showed that a few pigment granules had not reached the pigment zone, but had lingered near the equator of the eggs. The eggs treated with hypertonic sea-water showed no stratification. In some eggs one pole was slightly paler than the other, and fewer pigment-bearing granules could be found at this pole. But even in these eggs, the pigment was scattered throughout every part of the cytoplasm. At 11:29 the eggs in hypertonic sea-water were centrifuged again and compared with normal eggs given the same treatment. Longer and more vigorous centrifuging was resorted to, and the tubes of the centrifuge were revolved 186 times per second for 35 seconds. The normal eggs now appeared perfectly stratified; they were often elongated as a result of the treatment. The eggs in hypertonic sea-water usually showed only the beginnings of stratification, a tendency for the pigment to be massed toward one pole. In a few eggs, however, the pigment was practically limited to one half of the egg.

These experiments show a striking increase in cytoplasmic viscosity after eggs have been exposed to hypertonic solutions. This could be noted both by observation of the flow of the cytoplasm itself, as well as by more careful observations of the movements of granules through the cytoplasm. The only explanation of such a marked increase in viscosity is that gelatinization or coagulation has taken place within the egg. Undoubtedly exosmosis causes some constituent of the cytoplasm to change from sol to gel.

Endosmosis was also found to cause coagulation, either when it resulted from a dilution of the outer medium, or when it was the result of a lowered surface tension of the vitelline or plasma membrane. This could be demonstrated either by observing the cytoplasmic flow from compressed eggs, or by noting the granular movements in centrifuged eggs. Numerous experiments were made with distilled water. In an experiment of June 30, 1914, a square piece of glass which had been broken from a slide was used to compress the eggs. It weighed 1.31 grams. The piece of glass was held between the thumb and forefinger, so that its one edge rested on the slide just to one side of the coverslip which covered the eggs under observation. At the desired time, the glass piece was dropped and the compressed eggs could then be immediately observed. Normal eggs were deprived of their jelly by shaking them 6 or 8 times in a test-tube, and were then subjected to the pressure of the piece of glass as just described. The eggs all ruptured, the contents flowing out for a distance of about 60μ. Eggs (from the same female) were then dropped into distilled water at 10:28 A.M. At 10:28¾ any jelly which may have remained around them was removed by shaking the eggs. At 10:29½ the eggs were subjected to the pressure of the same piece of glass which had been used in the case of the normal eggs. They resisted the pressure and remained circular or nearly circular in outline. In the eggs observed, membrane elevation had not taken place. The greater resistance to pressure of the eggs treated with distilled water is an indication of their increased viscosity. The eggs tended to remain spherical, they showed a definite elasticity. Similar observations showing increased viscosity after treatment with distilled water, were made a number of times both in 1913 and 1914.

On July 25, 1914, at 3:59 P.M., 7 drops of an egg suspension were dropped into 15 c.c. of distilled water. The eggs in the distilled water were then hastily pipetted into one tube of the centrifuge, the other tube contained untreated eggs. The centrifuge was started at 4:00½ P.M., and for 25 seconds the tubes were revolved at a rate of 156 revolutions per second. The eggs in distilled water were examined as soon as possible (about half a minute later). They showed not a sign of stratification. Most of the pigment had been lost as a result of the action of the distilled water, but high power examination showed that the pigment-bearing granules still retained some pigment. These granules were scattered all through the cell. On the other hand the normal eggs showed the typical stratification. The viscosity of the cytoplasm had therefore increased enormously in the eggs treated with distilled water, so that the granules were prevented from wandering through it. In another experiment (July 22, 1914) the eggs were transferred back to sea-water before being centrifuged. At 3:51 P.M., 5 drops of egg suspension were added to 15 c.c. of distilled water. At 3:55 P.M., some of these eggs were removed from the distilled water and placed into sea-water again. The eggs thus transferred were the ones studied; they were placed into one tube of the centrifuge, normal eggs occupying the other. Beginning at 3:59½ P.M., the tubes were revolved 165 times per second for 30 seconds. Upon examination it was found that whereas the normal eggs were completely stratified, not a sign of stratification could be observed in the eggs which had been exposed to distilled water. Evidently endosmosis, following immersion in distilled water, leads to a gelatinization or coagulation in the cytoplasm.

This effect is also produced when endosmosis follows a lowered surface tension of the plasma membrane. A drop of egg suspension was stirred up with a drop of chloroform, and the eggs rapidly increased in diameter. When subjected to the pressure of a piece of glass broken from a slide, the eggs flattened out but remained perfectly circular in outline. The same result was obtained if toluol was used instead of chloroform. The eggs under pressure behave like eggs which have been coagulated by some typical coagulant, *e. g.*, $HgCl_2$. On the other hand, when the normal eggs were subjected to the pressure of the same piece of glass, the cytoplasm flowed out a considerable distance. The results obtained with the centrifuging method are still more convincing.

At 10:02½ A.M. (July 23, 1914), 2.5 c.c. of toluol were added to 2.5 c.c. of sea-water containing eggs, and the mixture was stirred thoroughly with a glass rod (in a Syracuse watch-crystal). At 10:07½ A.M., 7 drops of the egg-containing liquid were transferred back to normal sea-water. Into one tube of the centrifuge were placed normal eggs, the other contained eggs which had been treated with toluol for five minutes. The eggs were then centrifuged for 30 seconds at a rate of 152 revolutions per second. Upon examination, the normal eggs appeared perfectly stratified, whereas those which had been exposed to toluol showed not a trace of stratification. As a result of the toluol treatment the eggs increased their diameter about 2μ. The experiment was repeated with identical results on July 25, 1 c.c. of toluol being added to 3 c.c. of sea-water. In this case the eggs were exposed 5 minutes, and were then centrifuged 23 seconds at an average of 158 revolutions per second.

Saponin was also found to produce a coagulation within the egg. At 5:35 P.M. (July 23), 7 drops of an egg suspension were placed in about 15 c.c. of 0.2 per cent. saponin solution (in sea-water). At 5:40 P.M., the eggs were returned to normal sea-water. The eggs were then subjected to an exceptionally long centrifuging process. Beginning at 5:47 P.M., for 50 seconds they were revolved at an average of 166.5 revolutions per second. Normal eggs subjected to this centrifuging process were of course completely stratified. Of the eggs treated with saponin, only about 15–20 per cent. showed any signs of stratification, the remaining eggs were all totally unstratified.

In the experiments with saponin and toluol, the reagent produces a lowering of surface tension directly. With some reagents, membrane swelling occurs first, and the lowered surface tension thus produced results in endosmosis. In this case also, coagulation follows an increase in egg volume. On August 4, 1914, eggs were washed in 0.55 *M* NaCl solution and were then dropped into 0.55 *M* NaCl plus 1.5 c.c. *N*/10 NaOH at 10:59¼ A.M. These eggs were then placed into one tube of the centrifuge, and into the other were placed eggs which had been immersed in 25 c.c. of 0.55 *M* NaCl at 10:58 A.M. Beginning at 11:16½ A.M., the eggs were centrifuged for 17 seconds at the rate of 153 revolutions per second. When examined, the eggs in NaCl alone showed stratification, although the boundary between the various zones was not a sharp one. Gray cap, hyaline zone, granular zone, were distinguishable, but the pigment was not entirely restricted to the pigment zone. On the other hand, the eggs in the alkaline NaCl solution showed not a vestige of stratification, in every case the pigment-bearing granules were evenly distributed throughout the cell. In these eggs exposed to an alkaline NaCl solution, membrane swelling had taken place. Similarly when membrane swelling was induced by 1 per cent. albumen, coagulation followed the endosmosis thus produced. On August 4, 1914, at 10:40 A.M., some eggs were placed in a filtered 1 per cent. solution of egg albumen solution in sea-water. After about 50 minutes, some eggs in the albumen solution were put into one tube of the centrifuge, normal eggs were placed in the other. The eggs were then centrifuged at the rate of 143 revolutions per second for 20 seconds. The normal eggs all showed stratification, although the various zones were not sharply marked off from each other. Of the eggs in the albumen solution only a few were examined. Of these ten showed not a trace of stratification, one egg showed stratification, but on examination it was found that its membrane had remained unswollen. Pressure experiments also showed a great increase in viscosity after membrane swelling. Sodium iodide and 1 per cent. albumen were used in these experiments.

It is evident that both the reagents which cause exosmosis and those which produce endosmosis, cause gelatinization or coagulation of some substance in the *Arbacia* egg. Thus all parthenogenetic agents produce such coagulative change.[1] The experiments indicate that the coagulation occurs just as soon as water enters or leaves the cell. It is probable therefore that the effect is primary, and not the result of intermediate changes.

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[1] I have omitted mention of radium and ultra-violet rays as parthenogenetic agents. I have not worked with either of these methods, and so can only offer a theoretical interpretation. Judging from Loeb's description (*Science*, N.S., XL., 680 (1914)), ultra-violet rays produce membrane swelling and thus they no doubt induce endosmosis. Moreover both radium and ultra-violet rays are protein coagulants, according to Dreyer and Hanssen, C. R. Acad. Sci., CXLV., 234 (1907). Hardy has also decribed gelatinization of a globulin solution as a result of radium radiation. Proc. Camb. Phil. Soc., XII., 201 (1903).

In normal fertilization, the sperm likewise produces coagulative changes in the cytoplasm. Albrecht in 1898 showed that there was an increase in viscosity after fertilization. He drew his conclusions from observations on compressed eggs. I have repeated Albrecht's observations a number of times. Much more striking demonstration of gelatinization or coagulation after fertilization is afforded by the centrifuge method. On July 22, 1914, eggs from a single female were washed twice and divided into two lots. At 10:00 P.M., half of the eggs were fertilized. One tube of the centrifuge was then filled with fertilized eggs, the other contained unfertilized eggs. At 10:10 P.M., the tubes were revolved for 18 seconds at an average speed of 180.5 revolutions per second. The eggs were then examined immediately. The unfertilized eggs were typically stratified. On the other hand, the eggs which had been fertilized were not at all stratified. Out of hundreds examined, only one showed stratification and that was peculiar in lacking an elevated membrane. Probably it had escaped fertilization. The coagulative change begins to be apparent very soon after fertilization. In one case the beginnings of the process could be observed 2½ minutes after insemination. At 8:24 P.M. (July 26) some eggs were fertilized in a small volume of sea-water and were shaken about to insure rapid sperm contact. At 8:26½ P.M., fertilized and unfertilized eggs were centrifuged at the same time (in separate tubes). For 23 seconds an average speed of 141 revolutions per second was maintained. Upon examination the fertilized eggs showed some evidences of stratification. The gray cap was becoming evident in some. But most of the fertilized eggs showed little more than a tendency for the pigment to mass at one pole. The unfertilized eggs were clearly more stratified. They showed the gray cap plainly in all cases, and a hyaline zone was also recognizable in them. Thus only 2½ minutes after insemination, the sperm had already begun to produce coagulative changes in the cytoplasm.

From these experiments, I have been led to conclude that initiation of development, either artificially produced or the result of fertilization, always involves a gelatinization or coagulation with the egg. This coagulative change is evoked before the egg interior shows any other signs of the approach of development. The coagulating substance (or substances) is evidently very ready to coagulate. The ensemble of conditions within the egg is no doubt responsible for this unstable state. Only a slight change in salt concentration is then sufficient to bring about coagulation. The fact that both increase and decrease of salt concentration are effective, suggests that the protein involved is of the globulin type.[1]

The mitotic spindle probably arises as a direct result of the coagulative change. The actual explanation of how this occurs is not truly a problem of biology but a problem of colloid chemistry, for it has been shown (Fischer, '99) that coagulation of inanimate proteins can produce structures identical in appearance with the mitotic spindle.

IV. SUMMARY.

1. The unfertilized *Arbacia* egg consists essentially of fluid proteins, surrounded by a stiff membrane (the vitelline membrane), which is the plasma membrane of the egg cell. This membrane is a protein gel with little or no admixture of lipoids.

2. There are two types of cortical change, membrane swelling and membrane elevation. In the former, the membrane absorbs water and increases in thickness; in the latter, the normal process, it becomes lifted away from the egg surface.

3. The vitelline membrane only loses its semipermeable properties several minutes after elevation, and the increase of permeability that then ensues is best regarded as a result rather than as a cause of the process.

4. Artificial membrane elevation is produced only by substances which lower the surface tension of the vitelline membrane. This is explainable on the basis of a theory that considers the various forces at play on the membrane. After its surface tension is lowered, the forces exerted outward are stronger than

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[1] If the protein referred to here is, as seems most likely, the substance which forms the spindle fibers, the fact that it shows the properties of a globulin becomes of significance. For globulins are the proteins most closely associated with contractile processes, muscles consisting almost entirely of them.

those balancing them, and the membrane is as a result pushed away from the egg.

5. Cytolysis is not due to a simple swelling of egg proteins for these can be shown to coagulate rather than to swell, it is due to a continuation of the same process as that which results first in membrane elevation.

6. The sperm produces membrane elevation by lowering the surface tension of the vitelline membrane. This it accomplishes by causing the membrane to swell at the point where it strikes the egg.

7. Experimental evidence is presented to show that this membrane swelling produced by the sperm is due to the action of an acid.

8. The fact that potassium cyanide does not prevent initiation of development by hypertonic sea-water, is taken as evidence against the oxidation theory of artificial parthenogenesis.

9. All initiation of development involves the gelatinization or coagulation of some substance within the egg. This coagulative change can be demonstrated to take place before the egg interior shows any other signs of the approach of development.

The above summary covers only the more important points of the paper.

I desire to thank Professor F. R. Lillie, and also Professors Child and Tower, for their kind criticism of the manuscript. To Professor Lillie I am also indebted for the use of a table at the Woods Hole Marine Biological Laboratory, where all of the experimental work was done.

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ON SUCCESSIVE DUPLICATE MUTATIONS.[1]

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Nilsson-Ehle[2] was the first to formulate the hypothesis of duplicate factors or representatives for the same character. He brought forward evidence from crosses of red and white varieties in certain Swedish strains of wheat, tending to show that in different F_2 families plants with red and white grains occurred in the ratios respectively 3:1, 15:1 and 63:1; from which he concluded that three independent units for red were present, each of which could produce the color alone. Although his conclusions were criticized by Kajanus,[3] yet there remains a strong presumption in their favor, and several other cases of supposed duplicate factors have been described, though these have for the most part rested upon more insecure data than the original instances of Nilsson-Ehle.

Certain suggestions have been made concerning the origin of this duplicate or triplicate condition. Emerson and East[4] pointed out in general terms that if a factor should become located in a different chromosome or should be affected in any way so as not to be always allelomorphic to itself, then a duplication of determiners would result. Shull[5] has listed the supposed cases of duplicate determiners and remarks that such a condition of duplication might also result from "repeated progressive mutations." In the same paper, Shull endeavors to account for the origin simultaneously of a duplicate "gene" for capsule form in *Bursa bursa-pastoris* and, at the same time, of the mutant *B. Heegeri*. That hypothesis will not, however, apply to the probably more frequent cases in which duplicate factors for a particular character are found without any other mutation having taken place. An explanation will therefore have to be found for the duplicate or triplicate condition in wheat or in any other organisms in which it occurs.

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[1] Presented before the American Genetic Association, San Francisco meeting, August 3, 1915.

[2] Nilsson-Ehle, H., 1909, "Kreuzungsuntersuchungen an Hafer und Weizen," I., *Lunds Univ. Årsskrift.*, N.F., Afd. 2, Bd. 5, Nr. 2, pp. 122.

[3] Kajanus, B., 1914, "Zur Kritik des Mendelismus," *Zeitschr. f. Abst. u. Vererb.*, 12: 206–224.

[4] Emerson, R. A., and East, E. M., 1913, "The Inheritance of Quantitative Characters in Maize," Agric. Exp. Sta. Nebraska, Research Bull. 2, pp. 120.

[5] Shull, Geo. H., 1914, "Duplicate Genes for Capsule-form in *Bursa bursa-pastoris*," *Zeitschr. f. Abst. u. Vererb.*, 12; 97–149, Figs. 7.

It is the purpose of the present paper to discuss more precisely the manner in which such monohybrid characters originate and particularly the way in which they may afterward become duplicate or triplicate. *Œnothera rubricalyx* affords a typical case of a mutant originating as a monohybrid, probably through a transformation in one chromosome or one pair of chromosomes.[1] I have pointed out elsewhere[2] that when the duplicate or triplicate condition occurs it might be reasonably supposed to have arisen through the same general change having taken place independently in two or three different chromosomes of the x series.

In an original mutation of this kind the new character of course forms a pair by contrast with the old unaltered character. If a single chromosome has undergone this change and the new condition is dominant, then a heterozygous mutant Aa will be produced having the new character but splitting in its offspring in a 3:1 ratio. This is the way in which *Œnothera rubricalyx* originated from *Œ. rubrinervis*, as I have shown elsewhere.[1]

If now in the mutant race one or both members of a second pair, $a'a'$, of chromosomes undergoes a corresponding change, to A′a′, or A′A′ then we shall have duplicate factors AA′ for the same character, and in the offspring of such individuals the new type and the original type would appear in the ratio of 15:1. A similar mutation in a third pair would give the triplicate condition with a ratio of 63:1.

It may be pointed out that this assumption of similar changes in different members of the x series of chromosomes is by no means an improbable one. It does not assume that the chromosomes which underwent the change were alike, but merely that they were more nearly alike than the others of the series. That the chromosomes of the x series are actually differentiated there are many lines of evidence to show. One of the most recent is the fact, ascertained by Doncaster and Gray,[1] that in certain echinodern crosses particular chromosomes swell up and form vesicles in the strange cytoplasm of the egg or of another species while other chromosomes exhibit no such effect. On the other hand, the materials of the chromosomes obviously possess many similarities which probably outweigh their chemical differences.

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[1] See Gates, R. Ruggles, 1915, "On the Origin and Behaviour of *Oenothera rubricalyx*," *Journ. of Genetics*, 4: 353–360.

[2] Gates, "The Mutation Factor in Evolution," p. 317, Macmillan, London, 1915.

It may further be pointed out that if both members instead of one member of a pair of chromosomes underwent a change, say in a pollen mother cell, the only difference would be that four instead of two mutated germ cells would result, each of which might function in producing a mutant. It is almost impossible to determine whether one or both members of a pair of chromosomes underwent the change in any particular instance, but in either case the original mutant would be heterozygous, though continued inbreeding would produce ultimately a homozygous race, as in the case of *Œ. rubricalyx*. This is probably the history of Nilsson-Ehle's wheats which are duplicate or triplicate for the red color factor in their grains.

From the evidence obtained in F_2 and F_3 in crosses of various Swedish wheats having red kernels, with white-grained varieties, Nilsson-Ehle concludes[2] that while the varieties known as Sammet and Grenadier have 3 independent units for red, Extra-Squarehead has only one, since it gave (p. 67) only ratios approximating 3:1. In a later paper[3] continuing this work the same author finds (p. 22) that Swedish Binkel wheat contains two factors for red. From one F_2 family he grew 94 F_3 families, with results which may be tabulated as follows:

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[1] Doncaster, L., and Gray, J., 1913, "Cytological Observations on the Early Stages of Segmentation of *Echinus* Hybrids," *Quart. Journ. Micr. Sci.*, 58: 483–510, pls. 28–29.

[2] Nilsson-Ehle, H., 1909, Kreuzungsunterschungen an Hafer und Weizen. I. *Lunds Univ. Årsskrist.*, N.F., Afd. 2, Bd. 5, Nr. 2, pp. 122.

[3] Nilsson-Ehle, H., 1911, "Kreuzungsuntersuchungen an Hafer und Weizen," II., *Lunds Univ. Årsskrift.*, N.F., Afd. 2, Bd. 7, Nr. 6, pp. 82.

TABLE I.

Expected Ratio of Families.		Totals.	Expectation.
7	40 families were constant red		
4	23 families split in the ratio 3 : 1	483 : 142	468.75 : 156.25
4	25 families split in the ratio 15 : 1	789 : 47	783.75 : 52.25
1	6 families were constant white		

It will be seen that the frequency of families is very close to expectation. The totals of the families containing a 15:1 ratio are also very close to expectation, but for the 3:1 families the agreement is not so good. The evidence seems sufficient, however, to justify the conclusion that two factors and two only are here concerned.

In the same way evidence is obtained (p. 25) to show that a certain pure line (0406) has in one case a single factor for red and in another case two factors. To use the terminology of Lang, the race is monomerous in one case and dimerous in another. In crosses between the 0406 race and 0234, which was also red,[1] ratios of 15:1 and 3:1 were obtained showing that two factors were present, one of which must have been derived from each parent. Hence the 0406 race must in this case have been monomerous. In crosses between 0406 and a white race, 15:1 ratios were again obtained, showing that the 0406 race is now dimerous. The genetic relationships of the strains used in these two crosses is not stated, but a simple explanation is that in the meantime the strain had undergone a second (invisible) mutation.

No explanation of the origin of this condition was offered. But there are at least two ways in which the dimerous condition may have been derived from the monomerous: (1) Through a mutation on the part of a second pair of chromosomes, (2) through a re-mating of the chromosome pairs. Later we shall compare the consequence of each of these methods of deriving the duplicate condition. In the first case the duplicate mutation is produced by a change very similar to that which produced the original mutant. In the second case the secondary change is a mechanical one, very different from the primary change which was probably chemical in nature.

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[1] The results are given in *Ber. deut. bot. Gesells.*, 29: 65–69, 1911.

Another pure line of wheat (0290) was also found to be dimerous for the red factor in one case and monomerous in another. A race called 0501 was found to be probably trimerous like Swedish Sammet. Nilsson-Ehle considers it scarcely probable that in these two wheats the same three factors for red are present, and thinks that perhaps many more than three independent factors have to be reckoned with. There seems, however, no reason for such an assumption. It appears more probable that corresponding chromosomes undergo the same change in each case so that the factors are all homologous with each other, though of independent origin in the different races.

We may, therefore, account for the origin of the duplicate and triplicate "factors" for red in the Swedish wheats by assuming that successive mutations have occurred and that in each case the duplicate or triplicate condition has afterward become homozygous and stable through the repeated self-fertilization occurring in later generations.

Turning now to the history of *Œnothera rubricalyx*, it appears that the original monomerous condition has become dimerous in subsequent generations of culture. And it will be seen from the culture records that this has happened independently several times in different lines of descent.

It may be worth while first to recapitulate in briefest form the evidence for the original monomeric character of *Œ. rubricalyx*. The original mutant gave an F_1 offspring of 12 plants, 11 of which had red buds (R) and one green buds (r). Three of the former selfed produced F_2 families in which the ratios R:r were respectively 10:5, 14:6, and 33:11. The sum of these three families is 57:22 which is close to a 3:1 ratio and could not reasonably represent a 15:1 ratio, nor could either of the three ratios individually. Two plants descended from the F_2 family which yielded 33:11, a perfect 3:1 ratio were used to cross reciprocally with *Œ. grandiflora*, a green budded species of diverse habit. Since these plants were from a family which was obviously monomerous, the F_1 from the cross would either be all R (if the parent was homozygous) or R:r in equal numbers (if the parent was heterozygous). The numbers obtained were 30 R:28 r in one cross and 79 R: 71 r in the other. Hence the family which gave the ratio 33:11 was undoubtedly monomerous and up till that time a single mutation had taken place involving only one pair of chromosomes.

It was anticipated that the F_2 from *rubricalyx* × *grandiflora* and its reciprocal would again yield 3:1 ratios but it was found that in fact there were other ratios as well, the chief of which now appear to be 2:1, 4:1, 5:1 and 15:1. In my discussion of these extensive results[1] I was at first inclined to attribute them to an effect of the *grandiflora* parent in modifying the frequency of inheritance of the R character, and to conclude that since the cross with *grandiflora* had obviously modified the red-bud character R by dilution in many cases, it must also have modified the *frequency* with which R would appear. I have since grown a large series of F_3 families, the results of which are published in detail elsewhere.[2] In the present communication a further analysis of these F_2 and F_3 ratios will be made, from which it appears that the unexpected ratios obtained in these generations are probably not an effect of the cross with *Œ. grandiflora*, but they result in part from the subsequent occurrence of duplicate mutations in *rubricalyx*. Other ratios, such as 5:1 cannot, however, be fully explained in this way.

In this connection it seems desirable to point out that in the inheritance of any character there are two features to be taken into consideration: (1) The nature of the character, and (2) the mechanism of its distribution in the germ cells. Mendelian writers frequently ignore the former, and biometrical writers vitiate their case when they take no account of the latter; but in a complete account of the inheritance of any character both must be considered. As a matter of fact, although crossing with *grandiflora* probably does not modify the mechanism of transmission of R, yet it does seriously and permanently modify the character itself in some cases, as I have shown in previous publications.

We may now consider the ratios R:r in the F_2 and F_3 of *Œ. rubricalyx* × *grandiflora* and the reciprocal. A further study of these ratios makes it evident that they nearly all fall remarkably close to three or four ratios. So close is the fit that it seems probable that several ratios, such as 5:1, are significant as such, though at present no complete explanation of them can be offered. I was formerly inclined to regard some of these ratios as the expression of merely quantitative differences without

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[1] Gates, R. R., 1914, "Breeding Experiments which Show that Hybridization and Mutation are Independent Phenomena," *Zeitschrift f. Abst. u. Vererb.*, 11: 209–279, Figs. 25.

[2] "The Mutation Factor in Evolution," pp. 254 ff.

TABLE II.

F_2 (*Oe. grandiflora* × *rubricalyx*).

Ratios.	Expectation.	Agreement.	Conclusion.
68 : 16	63.00 : 21 67.20 : 16.8	3 : 1 possible 4 : 1 very near	4 : 1
142 : 15	147.00 : 10	15 : 1	15 : 1
133 : 4	128.40 : 8.6	15 : 1	15 : 1
Total 275 : 19	275.60 : 18.4	15 : 1 perfect Hence 2 families 15 : 1 1 family 4 : 1	
F_2 (*Oe. rubricalyx* × *grandiflora*).			
(a) 66 : 13	59.25 : 19.75 65.84 : 13.16	3 : 1 5 : 1 perfect	5 : 1
(b) 45 : 14	44.25 : 14.75	3 : 1 nearly perfect	3 : 1
(c) 47 : 3	Incomplete. In addition 9 dwarfs, 1 intermediate.		
(b) 134 : 44	133.50 : 44.5	3 : 1 perfect	3 : 1
(a) 67 : 13	66.70 : 13.3 60.00 : 20	5 : 1 perfect 3 : 1 unlikely	5 : 1
(a) 82 : 13	79.20 : 15.8 71.25 : 23.75 89.00 : 6	5 : 1 very near 3 : 1 very unlikely 15 : 1 " "	5 : 1
(a) 77 : 15	76.70 : 15.3 69.00 : 23 86.25 : 5.75	5 : 1 perfect 3 : 1 unlikely 15 : 1 very unlikely	5 : 1
45 : 14 134 : 44 (b) 179 : 58	177.75 : 59.25	3 : 1 *very* close	3 : 1
66 : 13 67 : 13 82 : 13 77 : 15 (a) 292 : 54	288.30 : 57.7 259.50 : 86.5 324.40 : 21.6	5 : 1 very close 3 : 1 unlikely 15 : 1 impossible[1] Hence in F_2 2 families 3 : 1 4 " 5 : 1 0 " constant	5 : 1

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[1] By "impossible" is meant that the chances against this interpretation, taken in connection with the other results, are so great that for practical purposes it need not be considered.

F_3 (*Oe. grandiflora* × *rubricalyx*).

Ratios.	Expectation.	Agreement.	Conclusion.
231 : 56	229.60 : 57.4	4 : 1 very near	4 : 1
	239.20 : 47.8	5 : 1 ?	
	191.30 : 95.7	3 : 1 impossible	
	268.10 : 18.9	15 : 1 "	
237 : 56	234.40 : 58.6	4 : 1 very near	4 : 1
	244.20 : 48.8	5 : 1 ?	
	219.75 : 73.25	3 : 1 improbable	
	274.70 : 18.3	15 : 1 impossible	
Total 468 : 112	464.00 : 116	4 : 1 very near	4 : 1
	483.30 : 96.7	5 : 1 improbable	
	435.00 : 145	3 : 1 improbable	
	543.75 : 36.25	15 : 1 impossible	
		Hence F_3 (*grandiflora* × *rubricalyx*) 2 families 4 : 1 Also 4 families constant R 3 " constant r 2 " intermediate in pigmentation of buds.	

F_3 (*Oe. rubricalyx* × *grandiflora*).

Ratios.	Expectation.	Agreement.	Conclusion.
57 : 31	58.70 : 29.3	2 : 1 very near	2 : 1
	66.00 : 22	3 : 1 ?	
	82.50 : 5.5	15 : 1 impossible	
2 : 3		?	
22 : 6	21.00 : 7	3 : 1 very near	3 : 1
112 : 69	120.70 : 60.3	3 : 1 impossible	
	135.75 : 45.25	2 : 1 near?	2 : 1 ?
12 : 2		?	
182 : 29	176.00 : 35	5 : 1 near	5 : 1
	168.80 : 42.2	4 : 1 impossible	
	197.80 : 13.2	15 : 1 impossible	
4 : 2		?	
55 : 2	53.40 : 3.6	15 : 1 close	15 : 1
37 : 2	36.60 : 2.4	15 : 1 perfect	15 : 1
72 : 6	73.10 : 4.9	15 : 1 very near	15 : 1
59 : 1	56.25 : 3.75	15 : 1 not very close	15 : 1
Total 223 : 11	219.40 : 14.6	15 : 1 very close	15 : 1
79 : 37	77.30 : 38.7	2 : 1 very near	2 : 1 ?
	87.00 : 29	3 : 1 ?	
	108.75 : 7.25	15 : 1 impossible	
5 : 3		?	
80 : 34	85.50 : 28.5	3 : 1 near	
	76.00 : 38	2 : 1 nearer	2 : 1 ?
		Hence in F_3 *rubricalyx* × *grandiflora* Probably 4 families 2 : 1 ratio 1 family 3 : 1 " 1 " 5 : 1 " 4 families 15 : 1 " Also 2 families constant for R 8 " constant for r 1 family intermediate 186 plants.	

more precise significance, but particularly the ratios 5:1 and 15:1 in addition to 3:1 fall so closely into definite categories that the probability of there all being significant is great. The foregoing table contains the F_2 and F_3 ratios for the various families.

Considering these data as a whole, the ratios nearly all fall remarkably close to whole numbers. In the first column of Table II. are given the actual ratios obtained, in the second column the expectation for different ratios, and in the third column the conclusion as to the ratios probably represented in each case. Many of the families are larger than these of Nilsson-Ehle, and in general they appear to fit the various ratios more closely.

Considering first the F_2 families, it will be seen that those whose ratios are 3:1 or 15:1 are in perfect or almost perfect accord with expectation. This being the case, it seems probable that the 5:1 and 4:1 ratios obtained are significant as such, and in any case they cannot be considered merely wide departures from 3:1. Of the 5:1 ratios the first, second, and fourth are in perfect agreement with 5:1 while the other one is very close, as is also the sum of these four families (292:54). The significance of these facts is further heightened by the fact that three of these four families (the first, third and fourth) are derived from selfing different flowers of the same F_1 plant. This is shown in my original record of these experiments,[1] and it almost forces the conclusion that in this particular plant as well as others giving similar ratios, R gametes were being produced with greater frequency than r gametes in the ratio 5:3. There is, however, another explanation which will be considered later.

In the results of Nilsson-Ehle, on the other hand, the ratios do not fall clearly into such intermediate categories but tend to form a continuous series of ratios as Kajanus pointed out. Thus in one series of crosses[2] between black and white glumes involving only monohybrid ratios, the F_2 ratios in the 13 families actually range from 2.2:1 (323:144) to 4.1:1 (230:56), yet the total (2468:795) is fairly close to 3:1. One of these families contained 86 black (B): 22 white (B). F_3 offspring were grown from each of these 108 plants and the results showed their composition to have been as follows: 36 BB:50Bb:22 bb. From this result Nilsson-Ehle concludes that there was a preponderance of "black" gametes over "white" ones. But a series of F_3 families in another cross gave the reverse condition, 26 BB:60Bb: 33 bb, from which the conclusion is drawn that white gametes were here more numerous than black ones. Even though these results offset each other yet they cannot be referred merely to chance fluctuations in ratios. But no further explanation of them was offered. It will be shown later that in my crosses of *Œ. rubricalyx* and *Œ. grandiflora* these deviating ratios do not offset each other but are all consistent with the hypothesis that R gametes are being produced with greater frequency than r gametes.

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[1] *Zeitschr. f. Abst. u. Vererb.*, II, p. 236.

[2] "Kreuzungsuntersuchungen," I, p. 18.

Returning now to Table II. the first ratio (68:16) is not a very bad fit for 3:1, although exceedingly close to 4:1. It might easily pass for 3:1 without further comment were it not for the fact that two other ratios in this table are in very close agreement with 4:1 while they depart very widely from 3:1. The sum of these two ratios (468:112) is very close to 4:1 while it is highly improbable as a 3:1 ratio, the more so since the actual 3:1 ratios are almost in precise agreement with expectation.

Among the three F_2 families from *Œ. grandiflora* × *rubricalyx*, two show a 15:1 ratio and one a 4:1 or perhaps a 3:1 ratio. Hence it might be supposed that the *rubricalyx* plant which was used as pollen parent, already possessed duplicate factors for red. But this was not the case. That plant was in fact heterozygous for a single factor, since when crossed with *grandiflora* it gave an F_1 of 79R : 71r, which is as near to equality as could be expected. The full history of the *rubricalyx* individuals used for this and the reciprocal cross has been given in pedigree form in another paper,[1] to which reference should be made. It may be said that in both cases they are descended from the family which contained 33R : 11r. One member of this family was pollinated by *nanella* and produced a family of 42 plants. One of the latter (No. IV., 2), which was a perfect *rubricalyx* in appearance but carried dwarfing latent, was used to pollinate *Œ. grandiflora*. Since the F_2 offspring of this cross gave 15:1 ratios in two families, while the F_1 was a 1:1 ratio, duplicate mutations must have intervened between these two generations. The two plants which were the parents of the families containing 142 R: 15 r and 113 R: 4 r respectively must have possessed the duplicate factor in all their germ cells, so that they were heterozygous for R and R′. Their composition might then be written RrR′r′.

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[1] Gates, *Zeitschr. f. Abst. u. Vererb.*, 11: opp. p. 216 and on p. 217.

As pointed out earlier in this paper, such a condition might have arisen (*a*) through the transformation of a chromosome belonging to a second pair, (*b*) through an exchange of mates on the part of two pairs of chromosomes. We may now examine the comparative credibility of these two alternatives. There are certain difficulties with either hypothesis, one of which is that the transformation from the monomerous to the dimerous condition, whether effected by chemical or mechanical means, must apparently have taken place early in the ontogeny, before definitive germ cells are formed. The alternative hypothesis would be that all the germ cells had undergone the transformation simultaneously and independently, which one cannot believe possible.

There is, however, one consideration which makes it appear probable that the duplicate condition for R is not usually arrived at through a transformation of a new chromosome, but rather through a redistribution of the chromosomes. The 15:1 ratio can only be obtained from an RrR′r′ parent, in which both duplicate factors are heterozygous. It would therefore be necessary to assume when a 15:1 family is derived from a 3:1 family, that a chromosome belonging to a new pair had undergone a chemical transformation while its mate and the mate of the original modified chromosome were unaffected, *i. e.*, that the condition Rrr′r′ became altered directly to RrR′r′. This is very unlikely. On the other hand, as I showed long ago,[1] the chromosomes in *Œnothera* are very loosely paired during the reduction division, and moreover irregular chromosome distributions have been shown to occur at this time (as in the production of *Œ.* mut. *lata*). I also (*l. c.*) pointed out the probability that exchanges of chromosomes of different pairs but without change in number would take place under these conditions. By such a mismating or exchange of mates on the part of two chromosome pairs, a plant which was homozygous (RR) for one factor would give rise to plants which were heterozygous for duplicate factors (RrR′r′). This is then what has probably occurred in the cases where plants from a 3:1 family have given rise to 15:1 ratios. The frequency with which such mismating occurs in *Œnothera* may thus be estimated.

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[1] "A Study of Reduction in *Œnothera rubrinervis*," *Bot. Gazette*, 46: 1–34, pls. 3, 1908.

It is known that the chromosomes of *Œnothera* are in pairs (doubtless of paternal and maternal origin) throughout the somatic divisions, and the paired arrangement is probably a feature of the first mitosis after fertilization. If, then, a plant which would have been homozygous for a single factor (RR) becomes transformed into one which is heterozygous for duplicate factors (RrR′r′) and so gives a ratio 15:1 in its offspring, the most likely assumption is that *at the time of fertilization* the two R chromosomes, instead of becoming paired with each other, each paired with another (r) chromosome. Hence in this case the regrouping of chromosomes probably occurred not during meiosis where it would have to occur simultaneously in all the germ cells, but as a feature of fertilization or the first mitosis of the embryo. It will be shown later, however, that matings of the chromosome pairs probably also occur during meiosis and so modify the 3:1 ratio. To sum up, it appears that when a 15:1 family is derived directly from a plant in a 3:1 family, the remating of the chromosomes must have occurred at fertilization or soon afterwards; but when, for example, a 4:1 or a 5:1 family is derived from a 3:1 family, this may be accounted for by a certain amount of remating of chromosomes during meiosis.

The method above described will also apply to the origin of duplicate and triplicate factors in wheat and is perhaps more probable than the successive chemical transformation of different chromosomes. There is, however, a method of testing between these two possibilities. If the duplicate condition arises through a regrouping of the chromosome pairs, then, as has been mentioned, a race or a plant homozygous (RR) for one factor will give rise to a plant heterozygous for two factors

(RrR′r′). On the other hand, if the chemical transformation of a fresh chromosome takes place in a homozygous monomeric plant (RR), then the dimerous individual derived from such a monomerous plant should have the constitution RRR′r′.[1] It would be possible to determine between these two alternatives by breeding tests. If the constitution of the plant is RrR′r′ its offspring should give a 15:1 ratio. If it is RRR′r′ they would all be red in F_1 and F_2. But plants having the former formula could also be produced by the mismating of chromosome-pairs during meiosis in RR plants.

If we now return to the table (p. 210) and examine the F_2 from the reciprocal cross (*rubricalyx* × *grandiflora*) we find a total absence of 15:1 ratios, showing that not only was the *rubricalyx* parent of this cross monomerous but its offspring remained so. The parent of this cross was a member (No. IV., 8) of the monomerous family 33:11. As will be seen from the table, two of the F_2 families from *rubricalyx* × *grandiflora* gave perfect or almost perfect 3:1 ratios. Four others gave 5:1 ratios, three of which were perfect and the other very close to expectation as already pointed out. I have at present no further explanation of these 5:1 ratios to offer, but it seems probable that their significance will later become apparent.

Ratios more or less in excess of 3:1 could be obtained from plants homozygous for one factor, if there was a tendency for mismating of the chromosomes in meiosis. But this will not account for the definiteness of the 5:1 ratios obtained.

Turning to the F_3 of *grandiflora* × *rubricalyx* the full data are given in my book (p. 255). Four families were constant for R, 3 constant for r, 2 families numbering respectively 283 and 20 plants bred true to an intermediate condition, and 2 families split in the ratio 4:1, as shown in the table (p. 211). The excess of R's in the last two families is a significant excess over 3:1, however it is brought about.

In the F_3 of *rubricalyx* × *grandiflora*, four families give ratios nearest 2:1, one family near 3:1, one near 5:1 and four very close to 15:1. Whatever the significance of the 2:1 and 5:1 ratios in these families, the appearance of 15:1 ratios in the F_3 of this cross is of much interest, since the F_2 contained no families which could reasonably be construed as containing duplicate factors, except the one having the incomplete ratio 47:3. Reference to the pedigree numbers[1] shows that the first two are derived from the F_2 family No. 60 in which the ratio is doubtful, the third is derived from selfing a plant in the F_2 family No. 62, and the fourth from selfing one in family No. 63. In these two families the ratios were respectively 67:13 and 82:13, both of which are shown (p. 210) to be very near 5:1 ratios. The appearance of these 15:1 ratios in F_2 from 5:1 families can be explained if we assume that independent duplicate mutations have occurred in the F_2 families 60, 62, and 63. This must happen as previously outlined, through a plant which is homozygous for one factor giving rise to a plant which is heterozygous for two; or in other words, through the rearrangement of a pair of homologous chromosomes so that they belong to different pairs.

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[1] We have already found it highly improbable that a plant Rr could be directly transformed chemically into RrR′r′, since we should anticipate that the chromosome r would undergo a mutation before the chromosome r′.

Another point which will be explained by the present hypothesis is the difference in the depth of color in homozygous red-budded races. Thus in the F_3 families 93 and 95,[2] containing respectively 280 and 312 plants, the latter were constantly darker red than the former. The latter family was doubtless homozygous for duplicate factors (RRR′R′), or at least RRR′r′, since the family from which it was derived yielded 15:1 ratios. The former family was on the other hand probably homozygous for a single factor (RR) and hence not so densely red-pigmented.

It will thus be seen that in several instances 15:1 families have been obtained from the offspring of 3:1 or 5:1 families. All such cases can be explained by assuming that a duplicate mutation has intervened. The original mutation by which deep red buds in *Œnothera* first appeared is an extremely rare occurrence, having occurred but once in all cultures of *Œnothera*. When, however, a chromosome has once undergone this change it is reasonable to suppose that other chromosomes in the same nucleus could without difficulty take on an analogous transformation. The whole mechanism is, however, at hand in the meiotic divisions, for transforming the original 3:1 ratio into a 15:1 by merely redistributing the chromosome pairs.

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[1] See "The Mutation Factor in Evolution," p. 256.

[2] *L. c.*, p. 255.

In concluding this paper it is desirable to compare the related but different results recently obtained by Honing,[1] with two varieties of *Canna indica* which are naturalized in Sumatra. One variety has green leaves while in the other the leaves have a broad red margin.

From the offspring of plants of the latter variety Honing obtained ratios red: green of 3:1, 9:7 and 27:37. The same ratios were obtained in crossing the two varieties. These ratios are accounted for by the hypothesis that the coöperation of three "factors" is necessary to produce the red margin. If these are located in chromosomes belonging to three different pairs, then the resulting ratio should be 27 red: 37 green, since the character can only appear in the presence of all three factors A, B, C. On the other hand, if all three factors are located in the same chromosome a 3:1 ratio would be obtained, while if two of them were in one chromosome and the third in a chromosome of a second pair, the ratio would be 9:7.

It was found that in certain cases plants in families having a 3:1 ratio gave rise in the next generation to a 9:7 or 27:37 family. In such cases one may assume that a mutation has taken place resulting in a redistribution of the determiners, the three which were present in one chromosome being rearranged so that they are in chromosomes belonging to two or three different pairs. So far as I am aware, this is the first experimental evidence that an actual rearrangement of the chromomeres in the chromosomes is one of the kinds of change which the nucleus may undergo, the case being somewhat different from Morgan's well-known phenomena of "crossing over" in *Drosophila*. Further experiments are necessary to test the nature of this evidence for the occurrence of mutations in which such a rearrangement of the nuclear material can take place.

SUMMARY.

Nilsson-Ehle was the first to show that duplicate and triplicate factors for red are present in certain strains of wheat. He found, moreover, that the same strain may be in one case monomerous and in another case dimerous for this character; and that while, for example, Grenadier wheat possessed three independent units for red, Extra-Squarehead possessed only one. The origin of the original "factor" for red may be accounted for in the wheats as in *Œnothera rubricalyx*, through the chemical transformation of one chromosome or a pair of homologous chromosomes. The duplicate condition for the character R may have arisen (1) through a chemical mutation in a second pair of chromosomes, (2) through a re-mating of the chromosomes (RR) forming a homozygous pair. The latter method is for various reasons the more probable.

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[1] Honing, J. A., 1915, "Kreuzungsversuche mit Canna-Varietäten," *Rec. Trav. bot. Néerlandais*, Vol. 12: Livr. 1, pp. 26.

Although the original *Œnothera rubricalyx* was a monohybrid and continued so for at least two generations, yet in subsequent generations involved in crosses with *Œ. grandiflora*, 15:1 or di-hybrid ratios were derived from the offspring of members of 3:1 families. This can best be accounted for by supposing that in a plant (RR) homozygous for one factor, a re-grouping of the chromosome pairs occurred. This re-grouping involves merely an exchange of mates on the part of the chromosomes RR so that they now belong to different pairs. The formula for the plant may now be written RrR′r′, *i. e.*, the plant is heterozygous for two independent units for red and its offspring will give a 15:1 ratio.

The second mutation, producing the duplicate condition for R, is thus probably a purely mechanical process, while the original mutation which produced the "factor" R is a chemical change of wholly different nature. It is possible that in some cases the duplicate and triplicate conditions also arise through the chemical transformation of additional chromosomes.

When a 15:1 family arises from a 3:1 or 5:1 family, as has happened several times in *Œ. rubricalyx* hybrids, it is necessary to assume that the regrouping or remating of chromosome pairs which led from the monohybrid to the dihybrid condition, took place at fertilization, or at any rate early in the ontogeny, and is then handed down to the germ cells by mitosis. The chromosomes are known to be paired in the somatic divisions, and it seems probable that the manner of pairing set up in fertilization continues in this case throughout the ontogeny, though this is not true for all organisms. Otherwise it would be necessary to assume that when a plant in a 3:1 family gives rise to a 15:1 family all its germ cells have simultaneously undergone a mismating of the chromosome pairs during meiosis, a highly improbable event.

In the F_2 and F_3 hybrids of *Œ. rubricalyx* and *Œ. grandiflora*, in addition to 3:1 and 15:1 ratios, 2:1, 4:1 and 5:1 ratios occur. The 5:1 ratios at least seem to be significant, indicating that R and r gametes are regularly being produced in the ratio 5:3, or that a certain amount of re-grouping of the R chromosomes is regularly occurring during meiosis.

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REACTIONS AND RESISTANCE OF FISHES IN THEIR NATURAL ENVIRONMENT TO ACIDITY, ALKALINITY AND NEUTRALITY.

MORRIS M. WELLS.

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I. INTRODUCTION.

The present paper is the first of a series that is to deal with the relation of fishes to ions in the natural environments. It is purposed to point out some of the close correlations which exist between the physiology of fishes and their behavior, and to present evidence concerning the importance of such correlations in biological investigation in general. The data presented in the following pages deal with the reactions and resistance of fresh water fishes to acidity, neutrality and alkalinity;

the discussion of the data shows that the phenomena outlined receive much support from the work of other investigators and that the environmental factors which are important to fresh water fishes are probably of importance to many, if not all, other organisms as well.

The investigation has been carried on at the University of Illinois, in Professor V. E. Shelford's laboratory. The work has been done in connection with another line of inquiry regarding the reactions and resistance of fishes to salts. The results of this second investigation will appear as the second paper of the series.

II. APPARATUS AND METHODS.

Two general types of experiments have been run, namely, reaction experiments, and resistance experiments.

A. Reaction Experiments.

This method of experimentation was devised by Shelford and Allee ('13) and may be designated as the "gradient method." In brief the procedure is as follows: A solution gradient is established in an observation tank, the fish introduced, and its movements graphed. The graph, together with notes taken at the time, makes up the experimental record. Fig. 1 shows the type of tank used. A similar tank was used by Shelford and Powers ('15) in their experiments with marine fishes. A black hood screens the tank, the movements of the fishes being viewed through slits in the front of the hood.

The tank has a plate glass front and is lighted by symmetrical lights placed above. A plate-glass cover fits into the top and rests against the surface of the water. This cover is useful in experiments with gaseous gradients as it lessens the vertical gradient due to escape of gas at the surface.

The water flows into the tank through the openings (inlets) in the ends, then toward the middle; at the middle the water from the two ends mixes, the water from each end drifting somewhat past the middle, thus forming the gradient. The water flows out through the exits (outlets) in the bottom and at the top of the tank. An experiment consists of first establishing the gradient, and then introducing the fish and graphing its movements.

In establishing the gradient the flow at each end of the tank was fixed at 500 c.c. per minute in practically all the experiments. The flow of tap water was regulated to 500 c.c. per min. at one end and to 400 c.c. per min. at the other. Then 100 c.c. per min. of a solution of treated water was introduced into the 400 c.c. flow from a mixing bottle (*M B*). The 100 c.c. flow was kept

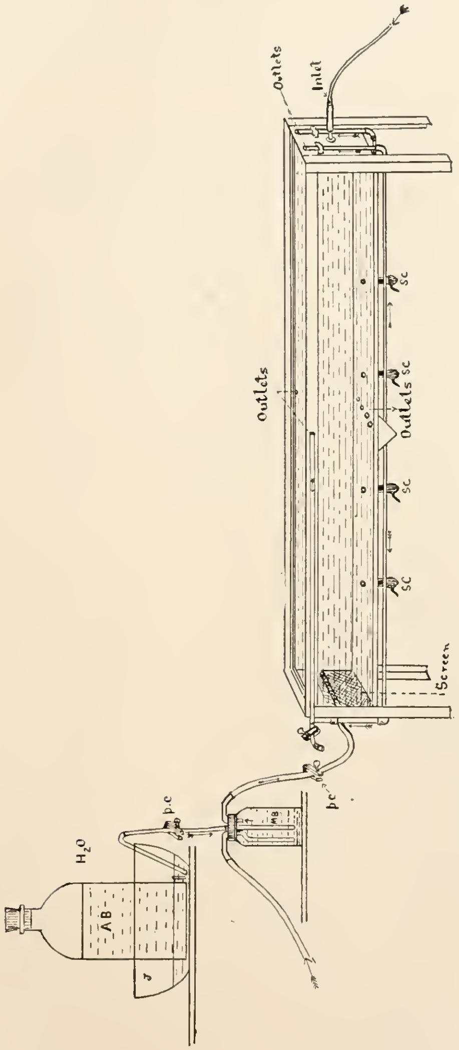

Fig. 1. Showing the gradient tank in which the reaction experiments were performed. The water flowed in at the *inlet* in each end, was distributed by the brass tee (*T*), drifted slowly to the middle, diffusing across somewhat, and out through the *outlets*. One fourth flowed out through each of the two outlets at the top and one eighth through each of the four at the bottom. The experimental factor was introduced from the aspirator bottle (*AB*). This bottle was filled with a solution five times as concentrated as that desired in the experiment. The solution was siphoned out of the jar (*J*) at the rate of 100 c.c. per min. into a mixing bottle (*MB*) into which tap water was flowing at the rate of 400 c.c. per min. This gave a flow into the tank, of 500 c.c. per min., and the desired concentration. Tap water, or water modified to a different degree, flowed into the opposite end at the same rate. Samples for testing were withdrawn through the stopcocks (*SC*) or from above by means of a pipette.

constant by using an aspirator bottle as in Fig. 1 (*A B*). This bottle was filled with treated water, corked at the top and placed in the jar (*J*). The water was siphoned from *J* and the pressure was constant as the solution escaped from *A B*, when the level began to fall in *J*. The strength of the solution in the aspirator bottle was always five times that desired in the treated water end of the tank.

The following variations of the simple graphing method of recording were used by Shelford and Allee and have been introduced here. (1) In experiments where the fish was decidedly inactive and remained in one end of the tank, it was driven into the opposite end with a rubber-tipped glass rod. The driving was done at regular intervals and was repeated at similar intervals in the controls. Dotted lines in the graphs indicate that the fish was driven. Experiments of this sort were few in number and have for the most part been thrown out. In some experiments, however, the fishes were active and yet remained constantly in one end of the tank. Driving was again resorted to, in some cases, to make sure that the selection of the given end was a reaction to the gradient. A return to the original end would indicate this to be the case. (2) A number of experiments was performed with 4–10 small fishes in the tank at the same time. These experiments were recorded by readings taken 30 seconds apart. The readings indicate per cent. of fishes in each third of the tank at the time of reading. (3) Usually the fishes were not placed in the tank until the flow at the ends had been going for some time. Thus a gradient was established before the fishes were introduced. In some cases, however, the fishes were either left in the tank when the ends were reversed, or introduced before a gradient had formed. The results of these experiments do not differ from the others except in per cent. of time spent in the thirds of the tank.

The controls were blank experiments, run with untreated water flowing in at both ends, or with no flow at either end. Experiments with the treated water first at one end and then at the other, also served as controls.

The gradient was determined by simple titration with standard acid or alkali, using phenolphthalein or methyl orange (or both) as indicator. The samples were collected by means of a pipette inserted to a given level below the surface, and the titrations were always made at once, with as much care as seemed necessary. For instance samples containing a high concentration of CO_2 need to be titrated with rapidity, while samples containing H_2SO_4 or KOH may be titrated without haste.

B. Resistance Experiments.

The procedure in the resistance experiments was very simple in most cases. In general the desired solutions were made up from standard solutions of the acid or alkali (measured from burettes) and the fishes introduced. Temperature was controlled by setting the jars containing the solutions, in running tap water. As the experiment proceeded, samples for testing were withdrawn when necessary, and the same amount of water was removed from the control. General controls were kept running throughout the entire time, while numerous temporary controls were set up as demanded by individual experiments.

The species of fishes used principally, have been the blue-gill (*Lepomis pallidus*), white crappie (*Pomoxis annularis*), green spotted sun-fish (*Lepomis cyanellus*), and bull-head (*Ameiurus melas*). Most of the fishes were caught (by seining) in the small streams in the vicinity of the university (the crappie came from a small artificial lake); all were brought into the laboratory with as little handling as possible, and placed at once in large aquaria. They were fed from day to day but fishes do not always eat well in confinement and as time went by they became more or less starved. The changes in the reactions of fishes, which accompany starvation have been investigated and will be discussed in another paper.

The chemicals used have been the chemically pure preparations of Kahlbaum or the analyzed preparations of Baker.

III. THE WATER.

An investigation of the reactions of fishes to salts in solution was begun at Chicago in 1912, at the suggestion of Dr. Shelford. In the fall of 1914 Dr. Shelford left Chicago to take a position at the University of Illinois and the writer accompanied him to continue the work in his laboratory at that place. The differences in the water supply of the two institutions brought up a number of new questions regarding the reactions of the fishes, and it was decided that to continue the investigation satisfactorily, more must be known of the effects of acid and alkaline water upon fishes and their reactions. This second investigation was therefore taken up and the results are published in advance of those with the salts, since they bear directly upon the interpretation of the latter. A brief comparison of the water of the two institutions will be profitable at this point.

The water at Chicago is pumped from Lake Michigan and analyses show it to be considerably different in gaseous and solid content from the water at Illinois, which comes from deep wells. In 1912 Allee, who had been working at Chicago on rheotaxis in isopods, came to Illinois, bringing with him a stock of animals. In his paper ('13) he compares the waters of the two institutions, and gives a table showing the differences in the dissolved content. This table is inserted here.

Table I.

A Comparison of Chemical Analyses of Chicago and University of Illinois Tap Water.

Analysis of solids in parts per million, and gases in cubic centimeters per liter.

	Chicago Tap.	U. of I. Tap.
Potassium, K	6.0	2.6
Sodium, Na	42.1	29.0
Ammonium, NH_4	0.04	2.3
Magnesium, Mg	11.3	34.9
Calcium, Ca	34.6	70.1
Iron, Fe	0.15	1.0
Aluminum, Al	0.00	1.3
Silica, Si	3.3	18.9
Nitrate, NO_3	1.7	0.7
Chlorine, Cl	12.0	3.5
Lead, Pb	0.01	0.00
Sulphuric acid, SO_3	0.04	2.3
Oxygen, O	10.46	0.12
Free carbon dioxide, CO_2	2.5	18.0
Half bound CO_2 (bicarbonates)	32.5	101.12

Allee states that the change of water did not greatly affect the rheotactic response of the isopods. At Illinois he kept the animals in aërated water, which was thus saturated with oxygen (5.5 to 7 c.c. per liter) while the free carbon dioxide was removed. If aërated sufficiently, the Illinois tap water becomes alkaline to phenolphthalein, and upon writing to Allee with regard to this matter he gives me permission to state that he kept his stock of isopods in such alkaline water for a period of 22 weeks, without increased mortality. He points out that the per cent. of rheotactic response, after a large number of trials, was 8 per cent. less than at Chicago but does not know whether or not to regard this as significant.

Table I. shows that Illinois water contains 18 c.c. of CO_2 per liter and practically no oxygen. Either of these conditions would alone prove fatal to fishes, while the combination would be doubly fatal (Wells, '13). Since aëration removes the CO_2 and at the same time saturates with oxygen, it was thought that this would fit the water for supplying the fish aquaria. The water as it came from the tap was therefore run through the aërating pans which form a part of the apparatus described by Shelford and Allee ('13). The device consists of a series of galvanized pans, placed one beneath the other. The water runs into the upper pan and trickles down through successive pans into a galvanized tank. From the tank, pipes lead to the aquaria. The flow into two large aquaria was regulated to 500 c.c. per minute for each and the fishes were now brought in from the nearby streams and placed in the aquaria in rather large numbers. The aquaria were 8 ft. x 2 ft.; about 300 small fishes were placed in each. This was overcrowding, but fishes have been kept successfully for some time, in closer quarters at Chicago.

The immediate mortality of the stock was not great. It was noted that the darters and other more sensitive fishes did not live well but the sunfishes, bullheads and minnows seemed to be normal. In a few days, however, these fishes began to die. It was thought that the water contained too large an amount of carbonates and an arrangement was made to introduce sulfuric acid into the aërated water at the galvanized supply tank. Enough acid was added to convert about one third of the carbonates into sulfates and with some benefit. It had been noted in the experiments that the fishes did not swim about as actively as usual and that their sensitivity seemed to be lessened, as they would swim into factors to which they are normally very negative. It was at this point that the study of the effects of acidity, etc., was decided upon.

Tests showed that the water entering the aquaria was practically neutral to phenolphthalein, varying a little from day to day. To determine the effect of the neutral water upon the fishes, a number was taken from the aquaria and placed in tubs of partially aërated tap water (water contained 6–10 c.c. CO_2 per l.). After a day or so in this water, they began to behave normally in the gradient again. The flow of water into the aquaria was now modified by diminishing the amount of aëration. The tap water was run down a wooden trough 12 ft. long, into the aquaria. This saturated it with oxygen but left it decidedly acid with CO_2. From now on the mortality of the stock of fishes was very low. The aquaria were not so crowded as at first, but that the decrease in the number of fishes does not explain the low mortality, will be brought out in experiments to be presented later.

The importance of the chemical reaction of the water to fishes had been foreseen (Wells, '13, p. 337) and it was decided that the peculiar properties of the Illinois water offered an excellent opportunity for continuing this investigation. At the same time it was thought that the work might perhaps throw some light upon a number of the reactions of fishes to salts, which seemed difficult to explain.

The advantages of the Illinois water are due to the following chemical properties: As it flows from the tap it is acid to phenolphthalein from the excess (18 c.c. per liter) of CO_2, and alkaline to methyl orange because it contains a large quantity (101 c.c. per liter) of bicarbonates in solution. The bicarbonates have been formed from carbonates according to the equation $CaCO_3 + H_2CO_3 \leftrightarrows Ca(HCO_3)_2$ and when carbonates are dissolved under the influence of excess of carbonic acid they are practically all converted into bicarbonate, the quantity of unconverted carbonate being negligible (Stieglitz, '09, p. 246, Seyler, '94, p. 105). Under the pressure in the water pipes, there exists an equilibrium between the carbonic acid and the bicarbonates, but when the water flows out of the tap, the pressure is removed and the carbonic acid at once begins to dissociate into CO_2 and water. The CO_2 passes off into the air and the dissociation of the acid continues until equilibrium with the CO_2 in the atmosphere is established. Parallel with the dissociation of the carbonic acid there goes an increasing tendency for the bicarbonates to break up to form the normal carbonate, and by the time the acidity from the carbonic acid has diminished to approximate neutrality, the bicarbonates are producing a sufficient quantity of the normal carbonate to give the water an alkaline reaction to phenolphthalein. Thus by regulating the amount of aëration, the water can be left acid, made neutral or even alkaline.

Biologists speak of the carbonates, bicarbonates, and carbonic acid, as fixed, half bound and free CO_2, respectively. The fixed is that existing as simple carbonates, the half bound that necessary to convert the carbonates into bicarbonates, and the free that remaining in excess (Seyler, '94, p. 104). It will be seen that the bicarbonates contain both fixed and half bound CO_2, *i. e.*, CO_2 which is to become half bound is added to CO_2, that is already fixed, to form the bicarbonates. Failure to recognize this fact often leads to confusion when these terms are used.

The amounts of the three kinds of CO_2 can be determined accurately by titration, using two indicators, phenolphthalein and methyl orange. Methyl orange is unaffected by H_2CO_3 and hence the bases present as carbonates or bicarbonates can at once be titrated with acid. Carbonates are alkaline to phenolphthalein, bicarbonates are neutral, and free CO_2 is acid. A carbonate titrated with acid, therefore, becomes neutral to phenolphthalein (if titrated under conditions which prevent loss of CO_2) when the carbonates have all been converted into bicarbonates.

Methyl orange is not affected by H_2CO_3 because this acid does not produce a high enough concentration of H ion. The indicator is however very sensitive to OH ion and reacts to the minute amounts that are present in a bicarbonate solution. Phenolphthalein, on the other hand, is very sensitive to H ion but not to OH ion. It therefore gives an acid reaction with CO_2 but is unaffected by the minute amount of OH ion which is present in solutions of bicarbonates.[1] Methyl orange will give an alkaline reaction in water in which the concentration of H ion is considerably greater than that of OH ion. Thus, in the presence of CO_2, titration with this indicator is not a determination of true alkalinity for the water is as a matter of fact acid, since it contains a higher concentration of H than OH ions. Marsh ('07) makes this error when he states (p. 337) that "the reaction of water which will support fish life must be slightly alkaline." His determinations were made with sulfuric acid, using methyl orange as indicator. The water to which he refers ("Potomac service water") was in all probability acid to phenolphthalein. Marsh also states that "when the water becomes even slightly acid, fishes cannot live in it." This would mean that fishes can not live in water which has been made slightly acid to methyl orange by the addition of an acid. I have added sulfuric acid to tap water until it gave an acid reaction to methyl orange, and find that fishes live in it as well as in the original tap water, *i. e.*, normally. The fishes should not be placed in such water until some little time after the adding of the sulfuric acid, however, for in the process of changing the carbonates to sulfates, a large amount of carbonic acid is liberated ($CaCO_3 + H_2SO_4 \leftrightarrows CaSO_4 + H_2CO_3$) and until this carbonic acid has dissociated and the CO_2 passed off into the atmosphere to a large degree, its presence will kill fishes which may be introduced. The amount of carbonic acid formed will depend upon the amount of carbonates in the water. (The reaction with bicarbonates will give the same result.)

In the following pages I shall introduce experimental data to show that fresh water fishes cannot live normally in water that is alkaline but that they require a certain degree of acidity to carry on their normal activities.

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[1] Noyes ('13) gives a table (p. 388) showing the acidity or alkalinity of solutions at the change of color for various indicators. Methyl orange gives an alkaline reaction when the OH concentration is only 10^{-9} while phenolphthalein is not affected until the concentration of OH ion reaches 10^{-5}. Methyl orange gives an acid reaction when the H ion concentration is 10^{-4} while phenolphthalein reacts when the H ion concentration is only a little more than 10^{-8}.

IV. PRESENTATION OF DATA.

The following experiments show the effect of different degrees of acidity and alkalinity, upon the reactions and longevity of fresh water fishes.

A. Reactions of Fishes to Acidity and Alkalinity.

1. *Reaction to Acids.*

(*a*) *To Carbonic Acid.*—A number of experiments was run to determine the reactions of the fishes to this acid. Three degrees of acidity were used for the most part: (1) Neutral, to very faintly acid (aërated water) (2) moderately acid (8–10 c.c. CO_2 per liter; obtained by using half and half mixture of 1 and 3); (3) strongly acid water (unaërated tap; 18 c.c. CO_2 per liter).

(1) *Moderately Acid Water vs. Strongly Acid Water* (*Graph 1, Chart I.*).—The fishes selected the lower acidity with much precision. They also spent much time at the surface, as is characteristic when the concentration of CO_2 is high.

(2) *Slightly Acid vs. Moderately Acid Water* (*Graph 2, Chart I.*).—The fishes were left in the tank, and the flow altered so that the moderately acid water ran into the end that had previously been strongly acid and neutral water was run into the opposite end. The fishes were graphed after five minutes. They definitely selected the end of the tank into which the neutral water was flowing. Test showed this end to contain 3 c.c. CO_2 per liter. Seven experiments with this combination were run and all gave similar results. Variations were due to specific and size differences. The larger fishes and the crappies and green spotted sunfishes selected a somewhat higher acidity than the smaller fishes, especially the blue-gills.

(3) *6 c.c. CO_2 per Liter vs. 4 c.c. per Liter* (*Graph 3, Chart I*).—The concentrations of CO_2 were obtained by regulating the amounts of aërated and unaërated water. Six experiments were run. The bullheads and blue-gills selected the lower concentration with precision, while the sunfishes and crappies chose the higher end with as much definiteness. Thus, as was seen in (2), the species differ in the optimum CO_2 concentration which they select at this time of year.[1] The difference in

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[1] Because of the fact that the resistance of fishes varies with the season (Wells, '14), it is very probable that the CO_2 concentration selected by a given species will show seasonal variations also. This point is yet to be investigated.

specific reaction may be in part a matter of size, as the crappies and sunfishes averaged larger than the blue-gills. Small sunfishes were, however, found to be less sensitive to CO_2 than were blue-gills of the same size. In this case the reaction is correlated with resistance as the sunfishes are more resistant than the blue-gills. The bullheads, however, are perhaps the most resistant of our fresh water fishes yet they are very sensitive to CO_2. The sensitiveness of the bullheads is probably related to the peculiarity of their integument which (Herrick, '02) has chemical perceptors "taste buds" scattered over its entire surface.

So far the fishes had for the most part selected the end of the tank containing the largest proportion of neutral water, *i. e.*, the lowest acidity. To ascertain definitely the reactions to the neutral water, the following experiments were performed.

(4) *Slightly Acid (3 c.c. CO_2 per Liter) vs. Neutral Water.)*—The slightly acid water was obtained by partially aërating the water which flowed into one end. This was done by running it through a galvanized tank which was a part of another piece of apparatus. The gradient tested practical neutrality at one end, and slight acidity at the other. The fishes definitely selected the end containing the CO_2 and were thus negative to the neutral water.

(*b*) *Reactions to Sulfuric Acid.*—It should be pointed out that experiments where other acids than H_2CO_3 are added to water, which contains bicarbonates, are open to misinterpretation if an attempt is made to compare the reactions of the animals to the acids, in this way. The addition of a strong mineral acid to such water does not result in the presence of a hydrogen ion concentration from the mineral acid itself, until all the bicarbonates have been decomposed (*i. e.*, changed to sulfates, etc.) or, in other words, not until the water has become acid to methyl orange. The reaction is a double decomposition, $H_2SO_4 + Ca(HCO_3)_2 \leftrightarrows CaSO_4 + 2H_2CO_3$. Until this reaction is completed, the chief result of adding the mineral acid will be to increase temporarily the concentration of carbonic acid by liberating the fixed and half bound CO_2. The concentration of H ion from this weakly ionized acid will be but a small per cent. of that which would have been obtained from the mineral acid itself; yet this will be the only hydrogen ion supply until the bicarbonates have all been changed to sulfate (if sulfuric acid is the mineral acid used). The tap water at Illinois requires approximately 90 c.c. of .1 *N* H_2SO_4 to neutralize the bicarbonates in one liter of water while the Chicago water requires about a third as much. According to the above equation one molecule of the acid liberates 2 molecules of CO_2. Therefore 90 c.c. of .1 *N* acid will liberate 210 c.c. of CO_2. Powers ('13) did not take this reaction into account and speaks of comparing the reactions of crayfishes in gradients of HCl and CO_2. As a matter of fact the amounts of HCl which he added to the Chicago tap water were probably used up in neutralizing the bicarbonates. Thus all his gradients were with carbonic acid, and the differences which he gets are due to the liberation of this acid in excess, in the reaction of the HCl and the bicarbonates.

(1) *H_2SO_4 to Neutralize all Bicarbonates vs. Neutral Water.*—The CO_2 liberated in this gradient made the water so acid that the fishes were soon overcome, and died if not removed from the tank. However at first they gave a decidedly negative reaction to the acid end.

(2) *H_2SO_4 to Liberate 40 c.c. CO_2 per Liter vs. Neutral Water.*—The fishes reacted much as they did in gradients of aërated vs. unaërated water. They were very negative to the acid end.

(3) *H_2SO_4 to Liberate 4 c.c. CO_2 per Liter vs. Neutral Water (Graphs 4 and 5, Chart I.)*.—Eighteen experiments of this sort were run. Of the 18 graphs, 14 show that the fishes spent 90 per cent. of the time in the acid half of the tank; 3 show more than 50 per cent. of the time in this half and 1 (small blue-gill) shows an 80 per cent. preference for the neutral end. That the fishes are negative to neutral water is thus confirmed. To ascertain the chemical reaction of the water at the point in the tank where the fishes turned back from the neutral end, numerous samples were titrated from this region, during the experiment. They showed that the water at this point contained about 1 c.c. of CO_2 per liter. The graphs shown in Chart I. are typical for all.

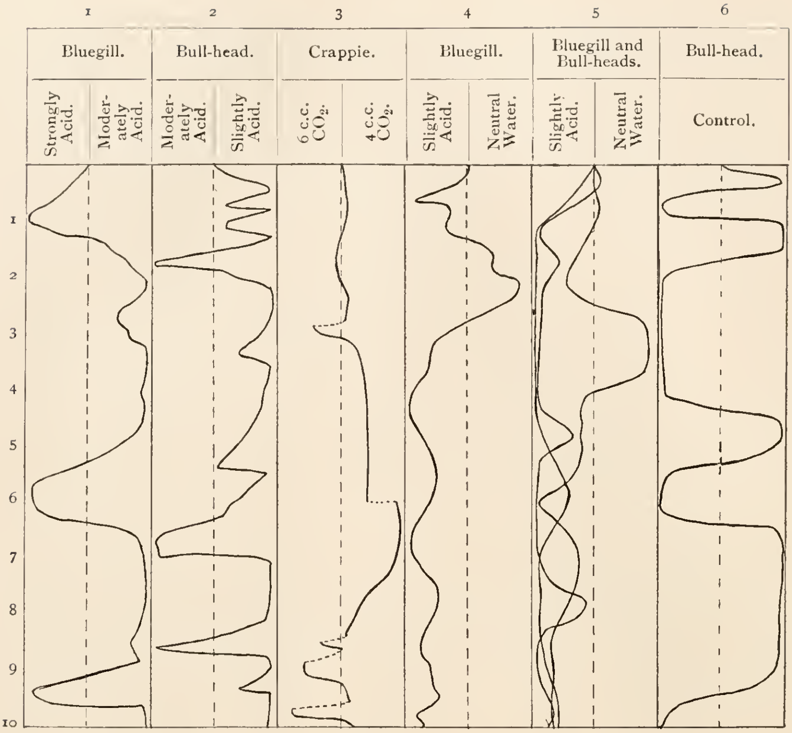

CHART I.

Showing the reactions of the fishes to different degrees of acidity. The gradient is between the two kinds of water, indicated at the top of each graph. Numbers at the left of the chart indicate time in minutes. Strongly acid = 18 c.c. CO_2 per liter; moderately acid = 8–10 c.c. per liter; slightly acid = 2–3 c.c. per liter; and neutral water = actual neutrality to 1 c.c. per liter. Dotted lines indicate that fish was driven.

(*c*) *Reaction to Acidity in Distilled Water.*—The distilled water, which was available in quantity from the chemistry department, was not rapidly toxic to the fishes and since the foregoing results are of some general biological importance, it was decided to repeat the experiments in distilled water. This water was faintly acid with CO_2 containing 2–3 c.c. per liter. It contained no salts; so the addition of a strong acid resulted in no complications such as those discussed in the case of the tap water. A number of experiments was performed with various strengths of acid and alkali. The neutral portion of the tank was kept track of by means of titrations and the reactions of the fishes to this neutral region especially noted. The results are presented in Table II.; in brief they are as follows. The fishes spent practically all the time in the acid portion of the tank, turning back from the alkaline end at a point just on the acid side of neutrality. They did not, however, select the highest acidity available, but swam back and forth in the tank between neutrality on the one hand and about $.0002N$ H_2SO_4 on the other. The small amount of CO_2 present in the distilled water may be neglected in the presence of the much more ionized acid. At the range of dilution used in these experiments, carbonic acid would have to be about 1,400 times as concentrated as sulfuric acid, to give an equal concentration of H ion.

TABLE II.

SHOWING THE REACTIONS OF FISHES TO ACIDITY AND ALKALINITY IN DISTILLED WATER.

Acid Used.	Concentration.	Reaction.
H_2CO_3	$.00004\ N$	Negative: choose higher acidity.
H_2CO_3	$.0001\ N$	Positive: some fishes choose this concentration in preference to either higher or lower acidity.
H_2SO_4	$.0005\ N$	Very negative.
H_2SO_4	$.0002\ N$	Still very negative.
H_2SO_4	$.00005\ N$	Positive when neutral water is the other choice.

The fishes used did not select alkaline water in any case except when the only other choice was neutrality. Then they spent most of the time on the alkaline side, rather than at the neutral point.

2. *Reactions to Alkalies.*

(*a*) *Alkalies in Neutral Water.* (1) *Na_2CO_3 (.01 N) in Neutral Water vs. Neutral Water.*—Six experiments were run with this combination. The results were rather indefinite. However, the graphs as a whole show a slight preference for the alkaline half of the tank. As has been pointed out already, the fishes are negative to the neutral water, and these experiments confirm this reaction, even though the only other choice is alkalinity.

(*b*) *Alkalies in Strongly Acid Water.*—In this water which is acid with CO_2 (18 c.c. per liter), the first action of the alkali will be to neutralize the acid. Thus a small amount of alkali introduced at one end will simply produce an acid gradient by lessening the acidity at this end. Eighteen c.c. of CO_2 equals an .0008 *N* solution. In most cases, the concentrations of alkali used have been much greater than this and the amount used up in neutralizing the acid may be looked upon as negligible. In some experiments, to be cited, the acid factor is of much importance.

(1) *Na_2CO_3 (.01 N) in Strongly Acid Water vs. Strongly Acid Water (Graph* 1, *Chart II.).*—The fishes stayed in the middle of the tank, coming to the surface very little. The gradient was acid at one end and alkaline at the other. Titrations showed that the fishes spent most of the time on the acid side of neutrality.

(2) *Na_2CO_3 (.002 N) in Strongly Acid Water vs. Strongly Acid Water (Graph 2, Chart II.).* Fifteen experiments were run with this combination. The graphs show that the fishes spent most of the time nearer the alkaline end than before, but titration showed that they were merely following the neutral point, remaining on the acid side most of the time.

(3) *Na_2CO_3 (.0005N) in Strongly Acid Water vs. Strongly Acid Water (Graph 3, Chart II.).* This concentration of alkali was just a little more than enough to neutralize the acid in the water of the alkaline end. The end was really slightly acid, however, from the diffusion of more acid from the acid end of the gradient. The fishes moved into the so-called alkaline (really slightly acid) end and remained there during the experiment. This was true for all the fishes used.

(4) *$NaHCO_3$ (.01N) in Strongly Acid Water vs. Strongly Acid Water.*—This salt is neutral to phenolphthalein as has been pointed out in the preceding discussion. A number of experiments, recorded both by graphs and readings at short intervals, were run with it. The results were not at all definite. The fishes seemed to be indifferent to this bicarbonate in acid water, or else they were not at all stimulated by its presence.

(5) *NH_4OH in Moderately Acid Water (Made it Faintly Alkaline) vs. Moderately Acid Water (Graphs 4 and 5, Chart II.).* Ten experiments with this alkali were run, to check up Shelford and Allee's work ('13) with it. They say (p. 252) that the fishes (*Abramis*) did not react to ammonia in a concentration which caused them to turn on their sides after an hour or more. In

my experiments, I found also that the fishes do not react to this alkali with the precision found for the other alkalies used.

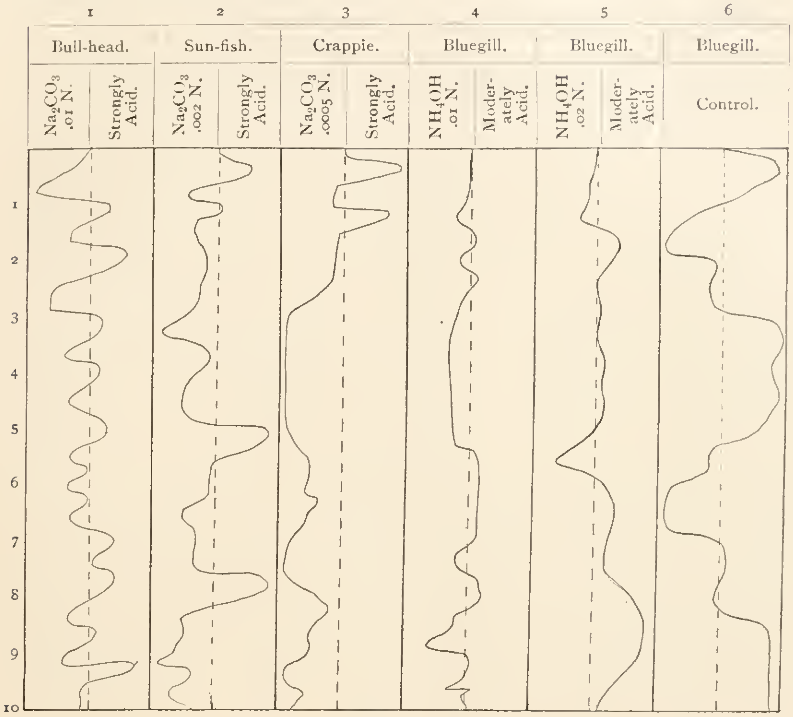

CHART II.

Showing the reactions of the fishes to alkalies. The gradient was between the two kinds of water indicated at the top of each graph. Strongly acid water = 18 c.c. CO_2 per liter; and moderately acid = 8–10 c.c. per liter. Numbers to left = time in minutes.

In the first experiments a .005 *N* solution was run into one end of the tank. The fishes selected the middle of the tank for the most part, though one blue-gill was positive to the ammonia end. The concentration of OH ion was of course very low, with so small a concentration of so weakly ionized a base, and since other experiments have shown that blue-gills are less negative to neutrality than are other fishes, this reaction is not surprising. The ammonia concentration was raised to .01 *N* and the fishes, blue-gill included, moved toward the tap-water end of the tank. Later the concentration of the alkali was raised to .02 *N*, but even now the avoidance of the alkali end was not nearly so definite as in the experiments with the other alkalies. Graphs 4 and 5 (Chart II.) show this indefinite reaction very clearly. In the .02 *N* gradient, the fishes were soon overcome by the toxicity of the water, which they selected, and they died there if not removed.

The fact that fishes fail to recognize ammonia in solution is of considerable importance, for this substance is being introduced into fish waters in many kinds of sewage. Furthermore it will be shown in the second paper of this series, that the gas has not lost its toxicity even when it has been converted into its various salts. The chemical explanation of the failure of the fishes to recognize and react to the presence of fatal concentrations of the hydrate in solution is probably due to the fact that the concentration of ammonia as gas, reaches a fatal concentration before the concentration of OH ion stimulates the fishes sufficiently to cause them to react negatively. They do not appear to react to the gas itself. Noyes ('13, pp. 203–4) states that ammonia dissolves in water, in part, without chemical change and that it is probable that a large part of the ammonia exists, as such, in the solution. He quotes Moore ('07) as calculating that only 30–40 per cent. of the ammonia exists as ammonium hydroxide, NH_4OH, at 20° C. Noyes thinks that the per cent. may be even less than this.

Again, the solution of ammonia diffuses through the water with great rapidity; much more rapidly than do most other substances. To determine the rate of diffusion, a little phenolphthalein was added to the aspirator bottle (*AB*, Fig. 1) containing the ammonia solution. The pink solution could be seen as it moved through the tank, and in less than a minute it had spread over the entire surface, and to a lesser extent, had penetrated the deeper water. Because of this rapid diffusion, no perfect gradient could be established with this substance. It may also be noted that ammonia behaves just opposite from the salts, the latter spreading along the bottom. In the ammonia experiments, the fishes seldom approach the surface, while in strong carbon dioxide gradients, they spend much time gulping the surface film. Shelford and Allee ('13, p. 231) state that in open tanks the amount of CO_2 at the surface is markedly less than at deeper levels.

3. *Conclusions Drawn from the Reaction Experiments.*

The reaction experiments recorded in the previous pages suggest the following conclusions. (1) Fresh-water fishes are negative to neutrality in favor of either slight acidity or slight alkalinity. Their normal choice is slight acidity (about .00005 *N* H_2SO_4 or .0001 *N* CO_2). (2) Species of fishes differ in the degree of acidity selected. Blue-gills select water that is but very slightly acid (1–2 c.c. CO_2 per liter, *i. e.*, .0001 *N* carbonic acid) while crappies select a concentration of from 4–6 c.c. CO_2 per liter. (3) The principal stimuli to which fishes react are H and OH ions. They do not react to ammonia, as a gas in solution.

B. RESISTANCE EXPERIMENTS.

It has been pointed out that the stock of fishes did not live well in the aquaria when these were supplied with water, which was neutral, or nearly so; to determine more exactly the reasons for the high mortality, between 50 and 60 experiments were performed. Some of these experiments lasted through a number of weeks, while others were finished in a few hours. The fishes were placed in different concentrations of acid and alkali in partly aërated water (from the aquaria) and in distilled water.

1. *Resistance to Acids.*

The resistance of fishes to carbonic acid has been worked out (Wells, '13) and it was decided to try the effects of other acids. Ten experiments with sulfuric acid in distilled water are summarized in Table III. The table shows that there is a concentra-

TABLE III.

SHOWING THE RESISTANCE OF FISHES (3 GRAM BLUE-GILLS) TO H_2SO_4 IN DISTILLED WATER.

Concentration of Acid.	Dying Time in Hours.
.001 *N*	3.5
.0005 *N*	7.0
.0002 *N*	42.0
.00015 *N*	60.0
.000075 *N*	Alive and vigorous at end of a month.

tion of this acid in distilled water, at which the fishes in question live as well as though in tap water. Higher concentrations of acid are fatal, the time required to kill the fishes being proportional to the hydrogen ion concentration.

2. *Resistance to Alkalies.*

In a .001 *N* solution of KOH in distilled water, a 3 gram blue-gill lived 4 hrs. and 25 min. In a .0005 *N* solution, a fish of the same size was alive at the end of 10 days. Titration at this time showed that the water had become acid to phenolphthalein from the CO_2 given off in the metabolism of the fish. The experiment was discontinued. To make sure that the fish in the first experiment had not been killed by the toxic potassium ion, another 3 gram blue-gill was placed in a .01 *N* solution of $NaHCO_3$ in distilled water. At the beginning, this solution was neutral, but it was expected that the bicarbonate would dissociate and the solution would become slightly alkaline from the carbonate thus formed. A blank control, containing the same amount of bicarbonate, but no fish, was run. The fish in the experiment died on the third day. Titration showed that the water had become .0009 *N* alkaline. The control was .001 *N* alkaline. Blue-gills therefore do not live well in water which is even very slightly alkaline.

3. *Resistance to Neutrality.*

The foregoing experiments, together with many facts recorded in the literature, suggested the possibility that the fact that it is neutral may have something to do with the toxicity of distilled water. Thirteen experiments were performed to test this possibility in a preliminary way. The facilities available did not make it possible to experiment with absolutely neutral water, but the results obtained are suggestive, as neutrality was approached very closely in some cases. Most of the experiments were performed with water that came from a copper still and will be referred to as once-distilled water. A few experiments were performed with a much purer water which was the once-distilled water redistilled in a better still and coming in contact with little copper. In neither kind of water could the amount of copper have been especially large, however, for small blue-gills lived in both kinds as well as in tap-water, so long as the water was slightly acid.

A comparison of the conductivities of the two kinds of water showed that the once-distilled had a conductivity[1] of 600×10^{-7} while the conductivity of the twice-distilled was only 10×10^{-7}. These conductivities are for 25° C. The conductivity of the water probably does not indicate the amount of copper present however for the metal is in all likelihood present in the colloidal state. Mengarini and Scala ('12) have shown that a number of metals, including copper, form a colloidal solution with distilled water even at room temperature, and especially in the absence of air. The conditions in a still would be especially favorable for the reaction, since the temperature is high and air excluded.

The addition of an acid to a colloidal solution would tend to precipitate the colloid, and this undoubtedly explains in part the effect of addition of acid in making distilled water less toxic,[2] as it will be shown that it does. Since, however, it has been shown (Bullot, '04) that distilled water which contains no copper is still toxic to organisms, other factors must be concerned. The evidence of the experiments presented in the present paper, indicates that the neutrality of the water is one of these factors.

It has been suggested in the preceding pages that the blue-gills and crappies differ in respect to the hydrogen ion concentration which they select and their resistance to the distilled water bears out this point as the crappies die in it in a day or so, while the blue-gills live indefinitely.

(1) *Experiments with Once-distilled Water.*—This water was slightly acid to phenolphthalein and was neutral to methyl orange. Its toxicity was tested by placing fishes in jars containing a liter of the water. A 12-gram crappie died in this

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[1] The conductivity of pure water is 1×10^{-7}.

[2] Locke ('95) calls attention to the fact that poisonous distilled water may lose its poisonous properties (if due to copper) by long boiling, and especially when brought into contact with sulphur, carbon, manganic oxide, cotton wool, silk, and other substances. The effect is very probably again due to the precipitation of the colloidal copper.

water in 2 days, but when this same liter of water was divided into two parts and a 3-gm. blue-gill placed in each part, both fishes were normal at the end of a month. In Table IV. is given a summary of a number of experiments performed with blue-gills in once-distilled water.

TABLE IV.

SHOWING THE RESISTANCE OF SMALL BLUE-GILLS (3–5 GRAMS) TO DISTILLED WATER THAT IS BARELY ACID WITH CO_2.

Conditions of Expt. Fish Placed in,	Resistance of Fishes.
1. Freshly distilled water	Normal after 5 days; expt. discont.
2. Boiled distilled water	Normal after 5 days; expt. discont.
3. Distilled water plus Na_2CO_3 to make neutral	Normal after 2 days. Water acid again.
Added a little $NaHCO_3$ to (3) to keep neutral	Dead on 10th day.
4. In dist. water as in (1)	Normal after 30 days.

Table IV. shows that the once-distilled water is not greatly if at all toxic to the blue-gills, but experiment 3 shows that these fishes cannot live in the water if it is slightly alkaline. This same distilled water is rapidly toxic to the crappies and sunfishes, however, as was shown in an experiment already described and in those which follow. This lack of resistance of the sunfishes in particular is a complete reversal of the ordinary specific resistance of these fishes as compared with the blue-gills, for in carbon monoxide, ethylene, sulphur dioxide, etc., the sunfishes are much more resistant than are the blue-gills.

On January 30, a liter of water (once-distilled) was made .00005 *N* acid with H_2SO_4 and another liter left as it came from the still. An 8-gram crappie was placed in each jar. The fish in the pure distilled water was dead in 12 hrs. while the one in the distilled water made acid, lived for 65 hrs. Several other experiments of this sort gave similar results, showing that the crappies cannot live in the neutral distilled water when it is pure, as well as they can when it is made slightly acid. It is very probable that sightly higher concentrations of acid than those used would have prolonged the lives of these fishes even more successfully than the .00005 *N* but as the stock of fishes was running low, these experiments were reserved for another time.

An experiment with small bullheads is very interesting. Normally the bullheads are perhaps the most resistant fresh-water fishes known. In the reaction experiments they selected a rather low concentration of hydrogen ion but were decidedly on the acid side of neutrality. In the pure distilled water a bullhead (4 in. long) lived 8 days; another in distilled water made .00005 *N* acid, lived for 20 days.

(2) *Resistance to Doubly Distilled Water.*—This water was less toxic to the crappies than was the once distilled water, as it contained less colloidal copper. It has been pointed out that the toxicity of the once-distilled water was lessened by the addition of acid, partly because the acid precipitated the colloidal copper. The experiments indicate further, however, that the neutrality of the water must be reckoned with also. This is again brought out, and more definitely, by a few experiments with the twice-distilled water. A quantity of this water was placed in a large bottle and a solution of barium hydroxid was suspended over it. At the end of a week the water was practically neutral. Two portions were taken in 500 c.c. Erlenmeyer flasks and a small bullhead (2.5 in.) placed in each. One portion was left neutral and the other made slightly acid with H_2SO_4. The fish in the neutral water lived 16 days and the one in the acid water 19 days. A few other experiments were performed with the twice-distilled water and all gave similar results. The stock of fishes was about exhausted, however, and further experiments were delayed until another time.

V. GENERAL DISCUSSION.

The fact that in natural bodies of water the chemical reaction of the water may vary from alkalinity through neutrality to acidity or the reverse, makes the practical importance of a knowledge of the reactions and resistance of fishes and other organisms to such chemical conditions an obvious one. From the experiments and discussion which have gone before, it is clear that water which gives an alkaline reaction to phenolphthalein for any length of time during the year, is undesirable as a home for most fresh-water fishes. On the other hand, marine fishes (Shelford and Powers, '15) with the exception of the anadromous species, probably would not survive in water which was even faintly acid. Since algæ and other phytoplankton forms (Birge and Juday, '11 and '12) may cause a body of water to become partially or wholly alkaline, through their ability to dissociate the bicarbonates, vegetation in fish waters assumes a line of importance heretofore little considered. The effects of sewage upon the acidity or alkalinity of natural bodies of water must also be reconsidered in the light of its possible injurious or beneficial effects due to its chemical reaction. Thus a large number of interesting and important questions suggest themselves.

The effect of the chemical reaction of the water upon the distribution of organisms promises much room for investigation. There is no doubt but that fishes recognize the difference between very faintly acid or very faintly alkaline, and neutral water. Henderson's work ('13), upon the mechanism which maintains a constant proportion of H and OH ions in the blood of animals, suggests the physiological reason for this extreme sensitiveness of the fishes. It is clear that even very small variations in the proportions of these two ions in the blood of the organism, are of grave importance, and we find in the blood a combination of gases and salts that makes such variations impossible as long as the animal is normal. The blood will maintain its normal chemical reaction (just on the alkaline side of neutrality) in the face of relatively large changes in the environment, yet we know that the mechanism breaks down when the change is either too great or too long continued (acclimatization is not considered at this time). The hyper-sensitiveness of the animals to the chemical reaction of the water, in the case of aquatic organisms, is another important factor in preserving the normal reaction of the blood, as the reactions of the organisms work in a way that causes them to turn back from concentrations of H or OH ion that would be detrimental. The delicacy and accuracy of these reactions are evidenced in the reaction experiments which have been discussed in the preceding pages.

The physiological effect of the acid, neutral, and alkaline water upon the organism very probably has to do with decrease or increase in the permeability of the exposed tissue cells (especially gills in case of fishes). Osterhout ('14) has shown that in plant cells alkalies increase the permeability up to death; acids however at first produce a rapid decrease in permeability, followed later by an increase which continues up to death. The concentrations of acid used by Osterhout were .001 *N* to .03 *N*. Very low concentrations such as those used in the experiments discussed here would very likely maintain a permanent decrease in the permeability of the cells, and the concentrations of acid in which the fresh-water fishes normally live, may thus protect the fishes by decreasing the permeability of their gills and preserving the normal reaction of the blood. Alkaline water, on the other hand, does not do this for fresh-water fishes, and they soon succumb in it. The results of Shelford and Powers ('15) indicate that the action of alkaline water upon marine fishes is to produce a normal permeability of the membranes and it may be that acid water would kill these fishes by decreasing the permeability below normal.

The effect of neutrality upon the permeability of tissues has not been worked out, so far as I am aware, but since fresh-water fishes, and probably marine fishes also, are negative to neutral water, it must be that such water exerts a marked effect upon the permeability, or some other physiological condition, in the gill membranes. The negativeness of organisms to neutral water indicates that they are either over-stimulated in such water, or under-stimulation sets up internal disturbances. Thus they may avoid it because of its non-stimulating character. It may well be that in neutral water, the normal chemical reactions do not go on, for acidity and alkalinity surpass all other conditions, even temperature and concentration of reacting substance, in their influence upon many chemical processes. Of all the catalytic agents, H and OH ions are by far the most important, and in their influence upon the stability of colloidal systems they are unapproached by other substances (Henderson, '13).

Birge and Juday ('11 and '12) attempt to explain the vertical distribution of the plankton in the lakes of Wisconsin and New York, upon the basis of relation to oxygen and food. This attempt has, it seems to me, met with little success, and they themselves point out many contradictions. According to their idea, the plankton forms must in many instances be reacting positively to concentrations of oxygen which are as small as .1 c.c. per liter, or even less. This supposition is contrary to all the experimental evidence regarding the reactions of aërobic fresh-water organisms to this gas. In an attempt to correlate the distribution of the zoöplankton with the chemical reaction of the water, I have gone over Birge and Juday's tables and figures, and have come to the conclusion that such correlation exists. Their data indicate in practically all of the lakes (in the summer condition) a point at some depth below the surface of the lake, where the organisms are more numerous than at any other depth. In many cases this rise is proportionately very high and is usually of small amplitude. Thus the large number of forms occurs in a rather limited region vertically. After the rise, there is a marked diminution in the number of forms and then again at a little greater depth there is another increase, smaller than the first, but still very noticeable in their curves. This increase is followed by a second diminution. The first diminution usually occurs in or near the thermocline where the temperature often shows a very sudden lowering. The oxygen supply sometimes falls off here also, but not always, and in the lakes to which I refer particularly, the oxygen supply is practically the same at all depths. A very important fact, however, is that the water in the region of the thermocline, *i. e.*, at the region of smallest numbers of plankton, is often neutral or very nearly so (summer condition). Above this region the water is alkaline, and below acid. From the data given in Birge and Juday's Tables XVIII. and XIX. ('12, pp. 602–608), I have compiled the following table (Table IV.) to show the relation of the zoöplankton to this neutral region. Birge and Juday's Table XVIII. is a record of temperatures and gas contents at the different depths; Table XIX. is an analysis of the plankton catches made in ten lakes. The records for a given lake were all made on the same day. Table XVIII. gives titration records which show that in three of the lakes at a definite depth, the water was neutral. Table XIX. gives the plankton collections at different depths in these three lakes, on the same day. Table IV. inserted below, is made up from a combination of the data found in the two tables; most of the data in Table IV. refer to the three lakes in question. In the instance of *Triarthra*, however, the data come from two other lakes, as this form does not occur in the three lakes from which the other data are taken.

TABLE IV.

SHOWING THE RELATION OF ZOÖPLANKTON TO NEUTRALITY.

The table is compiled from Birge and Juday's ('12) Tables XVIII and XIX, Most of the data are taken from their records for the three lakes, Canandaigua, Seneca, and Skaneateles. In these lakes the neutral depth was accurately located by titration of samples. The titrations and plankton collections were made on the same say. The data for the rotifer triarthra are taken from lakes Hemlock and Keuka as these are the only lakes in which this form occurs in the records. n.c. = no collection at this degree of acidity or alkalinity. The figures in the columns indicate the number of forms per cubic meter of water.

Name of Animal.	Alkalinity in C.c. per Liter of CO_2 to Make Neutral.			Neutrality.	Acidity in C.c. of CO_2 per Liter.		
	3-2.	1.5-1.	.5-.25.	0.	.25-5.	.75-1.	.1-1.5.
Pleosoma (R).......	3,925	0	0	0	0	0	0
Vorticella (P).......	12,250	0	0	0	0	0	0
Asplanchna (R)......	11,320	400	0	0	0	0	0
Dinobryon (P)......	43,700	19,130	42,800	0	0	0	0
Diaphanosoma (C)...	2,885	2,750	n.c.	260	0	0	0
Nauplei............	28,150	28,050	13,250	570	140	40	205
Diaptomus (Co).....	7,850	6,660	17,350	2,220	1,440	390	100
Conochilus (R)......	130	290	250	250	30	65	30
Anuroea (P)........	4,000	1,250	200	30	20	20	20
Cyclops (Co)........	13,775	7,620	7,620	25	30	0	5
Notholca (R)........	625	685	65	0	65	0	0
Daphnia (C)........	1,260	650	400	130	1,145	25	0
Ceratium (P)........	52,330	104,500	85,160	2,025	11,760	5,750	1,670
Polyarthra (R)......	12,350	1,620	2,350	160	1,190	1,240	40
Malamonas (P)......	0	n.c.	770,400	95,600	1,900	0	1,900
Triarthra (R).......	0	n.c.	0	n.c.	1,050	1,110	2,425

Letters in parenthesis after name of animal indicate the following. (R) = Rotifer; (P) = Protozoan; (C) = Cladoceran; (Co) = Copepod.

Table IV. shows (1) that all the zoöplankton forms are more numerous on either the acid or the alkaline side of neutrality, than they are at neutrality itself, *i. e., they are negative to neutrality;* (2) some forms as *Pleosoma* and *Vorticella*, are found only in the alkaline water; (3) others range between slight alkalinity and slight acidity but are never very numerous at neutrality and often (*Daphnia*, *Ceratium*, etc.) show an increase on either side; (4) a few forms (*Triarthra*) occur wholly on the acid side of neutrality.

The factors that regulate the distribution of the plankton in the lakes are undoubtedly numerous. The only certain way to determine them is to investigate experimentally the reactions of the animals to the factors concerned, both singly and in combination. To do this would be tedious but not especially difficult. As an index to the distribution of these forms, I believe that the presence and position of a neutral layer of water will be found to be important.

Besides the experimental data presented in the papers by Birge and Juday, the literature contains much other experimental evidence which bears directly upon the question of the toxicity of neutrality to organisms. Much of this evidence is found in connection with experiments upon the toxic effects of distilled water, and the action of salts in antagonizing this toxicity. In a series of papers published by Ringer and his students between the years 1883 and 1893 the question of the toxicity of distilled water was investigated and its reality apparently demonstrated. It was also shown that various salts are effective in neutralizing this toxicity, some being much more efficacious than others. In 1893 Naegeli showed that for Algæ (*Spirogyra*) at least, the toxicity of distilled water was due to contamination from the copper stills in which it was prepared. Locke ('95) confirmed Naegeli's results by showing the effect upon certain fresh-water animals to be due also to the minute amounts of copper present, and Ringer ('97) again taking up the subject reversed his former conclusions and confirmed those of Locke. Jennings ('97) found that *Paramœcia* live for weeks in distilled water. Moore ('00) says that young trout and tadpoles (unfed) live as long in distilled as in tap water, *i. e.*, several weeks. Lillie ('00) says that *Planaria maculata* will live in distilled water. Pure distilled water seemed then not to be toxic to fresh-water animals though apparently toxic to most marine animals. *Fundulus* eggs seem to be an exception among marine animals (Loeb, '99), as they can live in distilled water for weeks and still produce normal embryos. In 1903 Bullot after testing the effects of distilled water upon the fresh-water amphipod, *Gammarus* concluded that pure distilled water was toxic to this crustacean. Bullot's experiments were performed with great care; he considered and seemed to have eliminated the following possible toxic factors: copper, impurities from the glass, low oxygen, ammonia, and carbon dioxide. He found also that NaCl in small concentrations would neutralize the toxicity of the pure water to such an extent that the animals lived almost as well in an .00008 *N* solution of this salt as in the natural fresh water. The toxicity of pure distilled water, he concluded, is due to the lack of salts in solution. Peters in 1908 performed some very careful experiments to test the effect of pure distilled water upon protozoa. He came to the conclusion that distilled water which contains no salts, and which is changed often enough to prevent their accumulation from the metabolism of the animals, is rapidly toxic to these forms.

I have gone over the above papers and have found many statements which indicate that the presence of a certain concentration of hydrogen ion, was beneficial to the animals experimented upon. For instance, Ringer ('83) states that the distilled water which he used killed minnows, on an average, in 4.5 hours. He also says that the distilled water was very faintly acid; so faint was the acidity that he did not rely upon his own judgment but had others make the test also. However, he says, to prove that the acidity was not the cause of the death of the minnows, he took three liters of water and to one added 6 drops of 10 per cent. acetic acid, to the next 12 drops and to the third 20 drops. He then placed three minnows in each liter of acid solution. After 24 hours, the minnows were "quite natural" and he concluded, therefore, that the acidity could not have been harmful in the case of the distilled water. This conclusion of Ringer's illustrates the attitude taken by most authors with regard to the presence of acid in the water, that is, the acid is looked upon as a detrimental factor, to be considered negatively. So far as I have been able to read, the authors quoted above have taken little consideration of the possibility that the presence of a certain concentration of H or OH ion is essential to the welfare of animals.

This Ringer does not suggest, even though the minnows in the acid water were well on the way to live as long as any of the animals kept in salt solutions. In this same paper, Ringer notes that when he put a large number of fishes (up to a maximum) into a given volume of distilled water, they lived longer than one or two fishes placed in the same volume of water. He attributes this to the excretion by the fishes of inorganic salts, and does not take into consideration the carbon dioxide factor which would have increased the acidity of the water to many times that of the almost neutral distilled water. Again, in speaking of the salts which are best for preserving life in distilled water, Ringer states that the calcium salts are better than those of sodium and potassium, that $CaSo_4$ is better than $CaCl_2$ and that the phosphate of lime $(Ca_3PO_4)_2$ is much superior to all the other salts. This latter salt, he states, is decidedly acid, and he says ('86) "it is interesting to observe that though the circulating fluid with phosphate of lime gives a slight acid reaction to delicate blue litmus paper it will sustain contractility of muscle for hours." Thus a small hydrogen ion concentration seems to be beneficial, if not essential, to the continued life and activity of the organisms and tissues in question.

The question of the existence of a carbon dioxide optimum for animals has received considerable investigation with varying results. Ringer in 1893 investigated the influence of carbonic acid upon the frog's heart and concluded that free CO_2 in saline solution arrests cardiac contractility. He does not state what concentrations of CO_2 were used, but since he speaks of passing carbonic acid through the solution "for some time," his solutions were probably very acid. In a few experiments he neutralized the slightly acid distilled water which was used to make up the saline solutions, with NaOH, and noted that in this neutral solution, the contractions of the heart very soon became abnormal. Jerusalem and Starling ('10) review the literature regarding the importance of carbon dioxide for the ordinary functions of the body, and report a series of experiments to determine its influence upon the beat of the heart of the frog, tortoise and mammal (cat). They conclude that the CO_2 tension in the blood must be maintained at a certain height, if the pumping action of the heart is to be normally carried out. In their review of the literature they point out that their conclusions are in accord with those of Miescher, Haldane, Mosso, Henderson, and Bottazzi (see pp. 279–280). The lowest concentration which Jerusalem and Starling used was 2 per cent. of an atmosphere or about 20 c.c. CO_2 per liter. Their highest ran up to 200 c.c. per liter. Hooker ('12) tested the effect of carbon dioxide upon muscular tone and, in opposition to Jerusalem and Starling, concluded that this gas does not appear to be directly beneficial to tissues, except in case of intestinal muscle rhythm. He thinks it may be indirectly beneficial. Like most other workers upon this problem, Hooker used very high concentrations of the gas. His concentrations varied from 5 per cent. to 20 per cent. of the gas, in the atmosphere to which the solution bathing the tissue was exposed. Water will dissolve nearly its own volume of CO_2 and thus the concentration of carbonic acid varied from 50 to 200 c.c. of CO_2 per liter. The smallest concentration used would kill most fresh-water fishes in a short time.

Reuss ('10) worked upon the effect of CO_2 upon the respiration of fishes and concluded that it is an important one. The regulation according to him is through the respiratory center and not peripheral as Bethe ('03) believed. Shelford and Allee ('13*a*) note the extreme sensitiveness of fishes to CO_2 in gradients, and think the production of the gas as a product of the metabolism of the organism may tend to increase its external effect when the fishes come in contact with water containing it.

Bullot ('04) in his work with the fresh-water amphipod (*Gammarus*) noted, as did Ringer in the case of fishes, that the animals lived longer in distilled water when a number was present in a given volume, or in other words, when the volume of water per individual was small. He says: "If the amount of water falls below a certain limit, the animals will live the longer, the smaller the amount of water, provided the quantity does not fall below a certain minimum." In Table V. I have collected Bullot's data showing this point. The table shows that the relation holds for *both redistilled water and water distilled in copper alone.* The length of life in the water from the copper still is proportionately shorter throughout.

TABLE V.

SHOWING THE RESISTANCE OF AMPHIPODS IN DISTILLED WATER, WHEN EQUAL NUMBERS OF ANIMALS ARE PLACED IN DIFFERENT VOLUMES OF WATER, OR WHEN DIFFERENT NUMBERS OF ANIMALS ARE PLACED IN EQUAL VOLUMES OF WATER (COMPILED FROM BULLOT, '04, PP. 204–5).

Per Cent. of Animals Alive After 2 Days.

No. Animals.	Volume of Water.	Water.	
		Redistilled in Glass.	Distilled in Copper.
1	Same throughout	45%	Proportionately less throughout.
5		80%	
10		90%	

Time Required to Kill One Half the Animals.

Same throughout	5 c.c.	10 days	8 days
	20 c.c.	2.5 days	1.5 days
	50 c.c.	1.5 days	1 day
	100 c.c.	same	same.

In considering the possible importance of CO_2 as a factor in the toxicity of distilled water, Bullot states that the water which he used was very faintly acid to phenolphthalein, but not enough to injure the animals. He says: "We know from the works of P. Bert that, for cold-blooded animals like the frog, for instance, CO_2 is toxic only when its concentration in the air reaches 15 per cent. This corresponds to a solution of 15 per cent. of this gas by volume, as the water dissolves its own volume of CO_2 at ordinary temperature and normal pressure. This quantity is 350 times larger than the one which could be found in the distilled water." A 15 per cent. solution of CO_2 means 150 c.c. per liter of water. 1/350 of this is .42 c.c. In other words the distilled water used by Bullot was practically neutral, since the amount of hydrogen ion to be obtained from so small a quantity of so little ionized an acid as carbonic acid, would be almost negligible. In the gradient experiments cited in this paper, I have shown that certain fishes are negative to so small a concentration of CO_2 as 1 c.c. per liter, in preference for slightly higher concentrations. I have further shown that these fishes do not live as well in distilled water that is practically neutral, as they do in the same water made slightly (.00005 N) acid. Thus the existence of a hydrogen ion concentration optimum for these forms seems to be clearly demonstrated.

Peters ('08) makes no mention of the possibility of the neutrality of the distilled water which he used, having something to do with its toxicity, yet in a previous paper ('07) he recognizes the importance of the presence of a certain concentration of hydrogen ion for the existence of certain protozoa in hay infusions. On page 346, he says: "The data obtained indicate that, of the chemical conditions, the concentration of acid . . . is one of the chief factors determining the biological content and history of a culture."

From the data and discussion that have gone before, it seems certain that the chemical reaction of the water is a factor of marked importance in the life history of fresh-water animals. Some fresh-water forms are apparently positive to alkalinity as seen in the fresh-water lakes (Birge and Juday, *loc. cit.*) and others, that normally live in water that is acid with CO_2 are not killed by living in alkaline water (isopods). On the other hand, many forms, and probably most of the fresh-water fishes belong here, are always found in acid water if such be available, and these forms cannot live normally in neutral to alkaline water. Shelford and Powers ('15) have shown that marine fishes select the alkaline side of neutrality in a gradient, and in this difference in the behavior of the fishes, lies a key to the fundamental physiological difference in the organisms of these two habitats. Fresh-water fishes must live in the presence of an excess of hydrogen ion if their life processes are to be carried on in normal fashion. Shelford ('14) states that the carbon dioxide content of the water over the breeding grounds of fresh-water fishes should not average more than 1 c.c. per liter, nor exceed 5 c.c. during the summer months. This statement is probably wrong in limiting the average to 1 c.c. per liter for some fishes, as the green spotted sunfish and the crappies are negative to this small concentration of CO_2 showing a preference for slightly higher concentrations. Blue-gills, on the other hand, select a degree of acidity that is very little above neutrality. The CO_2 concentration selected by fishes probably varies with the season, and certainly with the salt content of the water in which they live. The variations of the CO_2 optimum in salt concentration will be discussed in the second paper of the series.

One thing is clear; the chemical reaction of the water should be known with accuracy, in all experiments with salts and gases in solution. A recognition of this fact will help to clear up some of the many contradictory results which have been obtained by various workers. It seems to be demonstrated beyond a doubt that the toxicity of distilled water is in part due to the absence or scarcity of inorganic salts, but it is also evident that the neutrality of such water may be an important factor in its toxicity.

VI. GENERAL CONCLUSIONS.

1. The chemical reaction of the water is an important factor in the reactions and resistance of organisms.

2. Fresh-water fishes select slight acidity in a gradient, when the other choices are neutrality and alkalinity. They choose slight alkalinity in preference to neutrality.

3. The CO_2 optimum for the different species of fishes experimented upon, varies from very close to neutrality for the blue-gill, to 6 c.c. per liter for the sunfishes and crappies. This is for the November to January condition. At other seasons and in other waters, the optimum probably varies somewhat. The optimum acid concentration for fresh-water fishes in distilled water is about .00005 N H_2SO_4.

4. The distribution of the plankton in the lakes of Wisconsin and New York (Birge and Juday, '11 and '12) shows a very interesting correlation with the chemical reaction of the water. There are fewer animals at neutrality than in the slightly alkaline and slightly acid water just above and below the neutral layer. Thus the forms are negative to neutrality.

5. The neutrality of distilled water is a factor to be considered in its toxicity.

VII. ACKNOWLEDGMENTS AND BIBLIOGRAPHY.

I am indebted to Professor V. E. Shelford for many helpful suggestions and numerous courtesies during the working out of the experimental data and preparation of the manuscript.

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A PROCESS OF TEMPORARY CHAIN FORMATION BY FRONTONIA.[1]

WALDO SHUMWAY.

In May of this year, while studying the protozoan fauna of a small pond in the New York Botanical Gardens, the writer observed what appeared to be a chain of four holophrya-like ciliates. A careful search of the culture made during the following two weeks revealed about a dozen more such chains before the species disappeared. During this time the writer attempted to obtain a pure culture of these interesting forms, with an idea to studying the nuclear changes involved. Although these attempts were all, ultimately, unsuccessful, it has seemed best to put on record what few facts have been observed. The data here given has been obtained from the study of the living material and some few preparations both *in toto* and sectioned.

The following stages in the formation of these chains have been observed; single individuals, two-cell chains and four-cell chains. No chains of three or more than four cells have been observed among the large number (over one hundred and fifty) observed.

The solitary individual is an exceedingly large (circa 300 micra) ovoid holotrichous ciliate, densely pigmented and filled with large alveoli, of some alloplasmic substance which stains deeply with nuclear dyes. The color is dark brown to black with transmitted light and light brown to white with reflected light. The mouth is anterior and lateral with rows of cilia which simulate two undulating membranes. There is a large lateral contracting vacuole. The cortical layer contains trichocysts. The macronucleus is large and oval, the micronuclei have not been observed in my material. The form is an active swimmer in the surface film of a large culture, but when isolated in Syracuse watch glasses sinks to the bottom and encysts. Details of this process are given below.

In cases where the individual neither dies nor encysts after isolation, it becomes transformed by clearing of the pigment, lengthening and flattening the general shape of the body until it resembles the well-known species *Frontonia leucas.* To this species, therefore, I have assigned my material, although I am not positive whether what I have observed is a new stage in the life-cycle of *Frontonia leucas* or a new species.

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[1] From the Zoölogical Laboratory, Columbia University.

The process of chain formation is inaugurated by the formation of a large transparent cyst through the exudation of some gelatinous material in which the surrounding zoöglœa becomes entangled in large quantities. Within this cyst the individual slowly rotates, all the cilia beating and the contractile vacuole pulsating regularly. In one individual followed under the microscope a single transverse division occured about forty-five minutes after the beginning of encystment, and half an hour from its completion. Nine hours after, the daughter cells were dead, still connected and inside the cyst wall.

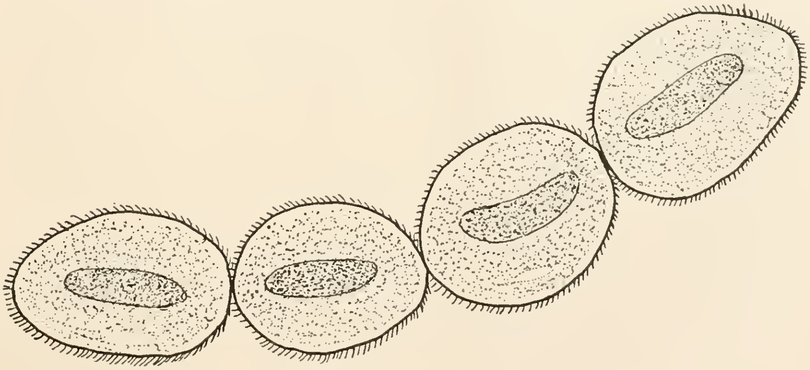

FIG. I. Chain of four cells, *Frontonia leucas.* 200 X. Outline drawn with camera from preparation fixed with sublimate-acetic and stained with picro-carmine. Details restored from free-hand drawings of living material.

Another encysted individual which had been isolated at the same time but which unfortunately had not been followed under the microscope had formed a chain of four cells. The writer has at different times observed in these cysts, single, double, and quadruple forms, as well as cysts containing two or four separate cells. One chain of four individuals was removed from its cyst for observation: it swam about for a time resembling an animated chain of beads, but finally broke up into its four constituent parts, which shortly assumed the typical *Frontonia leucas* appearance.

While the writer has not actually observed the process of transformation from the two-cell to the four-cell stage assumed above, he feels convinced from the facts cited as well as from sectioned material in which the macronuclei of the two anterior and the two posterior cells seem to have just undergone division, that these chains are formed by two successive transverse divissions without separation of the daughter cells and not by a single quadrupartite or by three successive terminal divisions as might be argued from *a priori* grounds.

Division preceded by encystment is not unknown among the free-swimming ciliates. In *Otostoma carteri* and *Amphileptus meleagris* according to Saville-Kent the divisions are sometimes multiple, but the daughter cells separate immediately after each division.

Chain formation too is not unknown among the ciliates, although heretofore it has been observed only among the astomatous forms parasitic in the digestive tracts of vertebrates. In these parasites, however, the chains are often of great length and are formed, so the weight of evidence appears, by a process of terminal budding. For an excellent discussion of these forms see Cépède (1910).

Jennings ('08) records a strain of *Paramœcia* appearing in his cultures which had apparently lost the power of separation after division. The general appearance and weak vitality of these forms, however point to the conclusion that Jennings was dealing with a weakened pathological race and not a genetic mutation.

The frequency with which these chains appeared, nearly ten per cent. of all the *Frontonia* observed, leads the writer to conclude that the phenomena described in this paper form part of a normal method of reproduction.

I have made attempts to raise these forms on hay infusion, rotten lettuce infusion (recommended by Popoff, '08), thyroid extract and the filtered water of the medium in which they were discovered. All were unsuccessful. I have been unable to maintain isolated individuals in Syracuse glasses of their normal medium longer than two days. For this reason I am unable to give a fuller account of the process under discussion nor carry out the experimental work suggested by it. The case however appears to be a unique instance of normal temporary chain formation among the free-living ciliates.

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SPERMATOGENESIS IN PARATETTIX.[1]

MARY T. HARMAN.

Wilson has said that "heredrity is a consequence of the genetic continuity of cells by division, and the germ cells form the vehicle of transmission from one generation to another."

If this be true we should look to the structure of the germ-cells fo· an explanation of the phenomena that have been and are being found out in heredity. Cytologists have discovered much concerning the structure of the germ-cells and the behavior of the chromosomes during the processes of maturation and division. The combined knowledge of sex and sex ratio, and the cytological constitution of germ-cells has shown in many forms, at least, a correlation between the inheritance of sex and the dimorphism of spermatozoa or eggs, or both. However, the vast amount of cytological work has been done with forms the behavior of whose characteristics in heredity is unknown. On the other hand, much of the work in heredity has been done with forms of which little or nothing is known of the structure of the germ-cells. It is the writer's good fortune to have access to material of which some of the ancestry is known for eighteen generations, covering a period of five years.

For a number of years Dr. R. K. Nabours[2] has been conducting experiments with regard to inheritance in *Paratettix*, a genus of the short-horned grasshoppers. The characteristics used in his investigations are the color patterns of the pronotum and femora of the jumping legs, and the lengths of the pronotum and wings. The data show that the inheritance of the color patterns is Mendelian in its behavior. In the F_1 hybrid no part of the color pattern of one parent species is ever replaced by the color pattern of the other parent, but the color patterns of both parents are present. Reciprocal crosses give identical results. The lengths of the wings and pronotum are not inherited but are closely correlated with the length of time required for the animal to reach maturity. These grasshoppers have furnished the material for the present paper.

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[1] Contribution from the Zoölogical Laboratory, Kansas State Agricultural College, No. 7.

[2] The writer wishes to thank Dr. R. K. Nabours for the grasshoppers which have furnished the material for this paper.

The work on the cytological constitution of the germ-cells of *Paratettix* has been undertaken for the purpose of discovering whether or not the microscope will reveal any differences in the germ-cells of very closely related forms which may be correlated with the differences in the color pattern. The spermatogenesis of only one form (*Paratettix leuconotus-leucothorax*) is given here. *Paratettix leuconotus-leucothorax* is a hybrid, obtained by crossing *P. leuconotus* with *P. leucothorax* (or by the interbreeding of two hybrids, one being a hybrid of *leuconotus* with some other form and the other a hybrid of *leucothorax* with some other form). No attempt has been made to show any relation between the structure of the germ-cells and the somatic structures. This will be discussed in a later paper.

The chromosomal complex of the spermatogonia of *Paratettix leuconotus-leucothorax* consists of thirteen rod-shaped bodies which may be divided into two groups—one group consisting of four larger chromosomes and the other of nine smaller ones. Neither the larger nor the smaller chromosomes form equal sized pairs as Sutton has found in *Brachystola magna* and which is so frequently described for the Hemiptera and is apparently characteristic of all Diptera. All of the large chromosomes and one of the small ones are bent rods or slightly U-shaped, but the other eight are almost straight. No one of these chromosomes has been surely identified as the accessory. However, in the early prophases there is always present a mass of chromatin which has a more compact consistency and stains more intensely than the remainder of the chromatin (*A*, Fig. 1). This mass has not been identified with any chromosome nor is it associated with a vesicle as described by Carothers for *Arphia simplex*. There appears to be no difference in the staining capacity nor in the compactness of the chromosomes in the late prophases (Fig. 2).

The spermatogonial spindle is long and slender, and has fine but distinct fibers which converge at the poles. The centrosome which is very distinctly visible in the metaphase stage is small and spherical. It stains almost as intensely as the chromosomes. The astral rays are short and indistinct. The chromosomes are at right angles to the spindle fibers in the metaphase stage (Fig. 7). A metaphase plate always shows one chromosome nearer the center of the spindle than any other chromosome. Sometimes it is completely surrounded by the others (Figs. 4 and 6) and sometimes merely one end is at the center of the spindle (Figs. 3 and 5). This chromosome is always one of the larger of the group of smaller ones but it is never the bent one. Few anaphase and no telophaase stages have been observed. Fig. 8 shows an anaphase with rather indistinct spindle fibers, which is characteristic of all the anaphase stages observed. The centrosome, which shows distinctly in the metaphase stage (Fig. 7), is now invisible. The chromasomes are no longer at right angles to the spindle fibers but are nearly parallel with them.

Fig. 9 illustrates the condition of the cell at the beginning of the growth period. The nucleus is large and comparatively clear. Some of the chromatin is in a finely reticular condition and stains faintly with iron-hæmatoxylin. However, a mass of the chromatin retains the compact consistency and the density of stain of the chromosomes (*A*, Fig. 9). It has a rounded form like a nucleolus. The boundary between the nucleus and the cytoplasm is quite evident. The nucleus continues to increase in size and the reticular chromatin, which now has a greater staining capacity, forms a thread or threads having a woolly appearance. There is no polarization of the loops of the spireme but they occupy almost all of the space of the nucleus and form an irregular tangled mass. The nucleolus remains at one side of the nucleus and does not have the woolly appearance that the spireme has (Fig. 10).

In the synezesis or contraction stage, the spireme seems to shrink away from the nuclear wall, leaving a clear space between the cytoplasm and the chromatin material. There is little difference between the character of the chromatin and its staining capacity in this stage and the preceding one. The compact mass of chromatin never loses its identity and always remains at one side of the nucleus (Fig. 11). The boundary of the nucleus soon becomes irregular, and the chromosomes of the primary spermatocyte is formed by a breaking up of the spireme thread into segments. The compact intensely staining mass which has been traced through the growth period is shown in Fig. 12 as a chromosome which differs in shape from the other chromosomes. It is ovoid and without a constriction in the middle, while all the other chromosomes are dumb-bell shaped. Not all of the chromosomes are formed at the same time. The chromatin retains its loose woolly appearance, until after it has broken up into parts, then it gradually becomes more compact, takes the stain more readily and each part assumes the characteristic dumb-bell shape. While this is taking place the boundary between the nucleus and the cytoplasm becomes more irregular and by the time the chromosomes are completely formed the cytoplasm has formed a vesicle around each of them (Figs. 13 and 14).

The chromosomal complex of the primary spermatocyte consists of six dumb-bell shaped chromosomes and one ovoid chromosome. Of the six dumb-bell chromosomes two are decidedly larger than the others and one of these is much larger than the other one, as is shown in Figs. 13 to 16 inclusive. The ovoid or accessory chromosome is never among the other chromosomes but always lies near the periphery of the nucleus as it did in the growth period. When the chromosomes have become arranged on the spindle the dumb-bell chromosomes are well toward the center of the spindle, while the accessory is always near the periphery. It does not remain long in the metaphase plate but soon passes toward one pole undivided much in advance of the other chromosomes. For this reason many sections of metaphase plates show only six chromosomes and those which show seven are often cut obliquely. Not all of the chromosomes in the primary spermatocyte divide synchronously. Fig. 20 shows five of the dumb-bell chromosomes divided while the largest one shows little constriction. This division is transverse as is shown in Figs. 16 and 20. There are no loops, rings, or U's which would give the least indication of a longitudinal division. In the metaphase or early anaphases the centrosome is a small spherical body and takes the stain readily. The spindle fibers are fine but distinct. The astral rays are similar to those of the spermatogonial divisions. In the late anaphases the centrosome is no longer visible and the spindle fibers are indistinct. There seems to be no resting stage between the first maturation division and the second maturation division.

The chromosomes of the second spermatocyte are ovoid. Metaphase plates show six and seven chromosomes (Figs. 23 and 24. The accessory cannot always be distinguished from the other chromosomes. It is either the second or the third largest. It divides in this division and passes to the poles in advance of the others (Fig. 25). In the late anaphases all the chromosomes have coalesced, although the number may yet be distinguished (Fig. 26). By the time the chromosomes have reached the poles they form a diffuse mass of chromatin at each pole, and the cell has begun to constrict in the middle.

The centrosome which is similar to the centrosome of the primary spermatocyte has disappeared. The spindle fibers have become indistinct. As the constriction of the cell is completed the chromatin has migrated to the center of each daughter cell.

In the changing of the spermatid into the spermatozoön two things are conspicuous from the beginning, the changes in the character of the chromatin and the elongation of the cell body. From the diffuse irregular mass there is formed an ovate body with the chromatin in a coarsely reticular condition largely around the periphery of it. The cell becomes elongate and larger at one end than at the other. The cytoplasm has changed from the tangled network to fibrillar strands of granules extending longitudinally across the cell. The cell wall is quite distinct. This condition is illustrated in Fig. 29. The spermatid continues to elongate. The nucleus becomes spherical and remains at one end of the spermatid. The more granular cytoplasm lies toward the periphery of the tail-like elongation. There is a portion of the cytoplasm extending from the nucleus through the center of the tail which is more finely granular than the remainder and takes the stain less readily. Part of the chromatin has become massed together, forming a sphere situated to the side of the nucleus near the end of the lightly staining area of the tail. The greater part of the remaining chromatin is distributed around the periphery of the nucleus (Fig. 30). As the tail becomes longer it becomes thinner and a filament extends from the nodule of the head through the entire length of the tail. Very little cytoplasm now surrounds the head (Fig. 31). Finally the head becomes arrow shaped and stains very intensely. The tail is long and filamentous and stains a little less intensely than the head. The head and a portion of the tail are illustrated by Fig. 32. The tail is more than four times as long as is shown in the figure.

McClung ('14) says: "It seems very evident that in the spermatogonia of the Acrididæ we are dealing with a chromosome complex of a very definite and precise organization which, in the great majority, presents itself without essential variation in number, size and form, fiber attachment, arrangement in the metaphase and behavior during division of its elements. *Stenobothrus* and *Pamphagus* seem to be definite exceptions in some of these respects. . . . " And again he says:

"With the exception of the *Stenobothrus*-like species, and *Pamphagus*, the students of the Acrididæ have reported a reduction of the 23 spermatogonial chromosomes to 12."

All of the genera of the family Acrididæ[1] discussed in McClung's paper belong to the three subfamilies, Tryxalinæ, Œdipodinæ, and Acridinæ, and none belong to the subfamily Tettiginæ. It would seem that with the general agreement of the great numbers of genera of this family which he and his students have studied as well as those of other independent workers that he would be justified in making the general statements concerning the family. However, *Paratettix leuconotus-leucothorax* of the subfamily Tettiginæ, show some exceptions. The spermatogonial number of chromosomes are thirteen instead of twenty-three. The number of chromosomes in the primary spermatocyte is seven. This the writer has found to be true also for both the parent forms of the hybrid as well as for others belonging to the genus *Paratettix*.

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[1] The writer is aware of the fact that there has been much shifting about of names of the short-horned grasshoppers and that some taxonomists consider the the grouse locusts of family value. If this should be the position which McClung takes, then he would not consider *Paratettix* as belonging to the family Acrididæ and it would follow that the observations recorded in this paper would not be exceptions to his statements concerning Acrididæ.

Robertson ('15) says: "In the Tettigidæ (Tettiginæ) a subfamily of the short-horned grasshopper family Acrididæ, I have found for all the specimens of at least four different genera which I have examined the number of chromosomes to be uniformly 14 in the female and 13 in the male."

From the above data one would scarcely be justified in saying that the characteristic number of spermatogonial chromosomes of the subfamily Tettiginæ is thirteen but the writer feels justified in saying that this is an essential variation in the number of chromosomes given in the above quotation from McClung as the number characteristic for the family Acrididæ. The writer has found no indication of multiple chromosomes.

In the prophase tetrad six of the chromosomes are always dumb-bell-shaped and one ovoid. There are none of the irregular shaped chromosomes as described by McClung and no indications of the annular chromosomes which he says "that practically without exception every investigator of recent years who has made a careful study of the maturation stages in the Orthoptera has seen and figured." If the dumb-bell-shaped chromosomes are similar to his I-shaped chromosomes they differ in that they do not have an enlargement in the middle, but rather they have the appearance of a constriction. This constriction is not due to the initiation of the division, for it is present before the chromosomes are arranged on the spindle; in fact, they have their characteristic shape before the spindle is visible.

The presence of a mass of chromatin in the resting stage of the spermatogonial divisions which is of a different form and different staining capacity and also the presence of a similar mass in the growth period, which can be identified as the accessory chromosome of the spermatocyte, gives added evidence for the continuity of chromosomes as definite entities.

The spermatozoön of *Paratettix leuconotus-leucothorax* is very different from the spermatozoön of *Paratettix cuculatus* as described by Hancock. He describes and figures the head of *P. cuculatus* as being small, thin, and acutely pointed. In fact, from his figure one would think that the head is very little thicker than the tail. He says that the middle piece is formed into a high, rather short, protoplasmic keel. No keel has been observed as forming a part of the middle piece of *P. leuconotus-leucothorax*. The head is many times thicker then the tail and is decidedly arrow shaped. The middle piece seems to continue from the head to the long filamentous tail without a definite dividing line between them.

Summary.

1. *Paratettix leuconotus-leucothorax* has thirteen spermatogonial rod-shaped chromosomes, four larger and nine smaller ones.

2. Neither the larger nor the smaller chromosomes form equal sized pairs.

3. The four larger chromosomes and one of the smaller ones are bent rods, the others are almost straight.

4. Neither spermatogonial chromosome has been surely identified as the accessory chromosome.

5. In the growth period is a mass of chromatin which is more compact and stains more intensely than the remainder of the chromatin. This is the accessory chromosome of the first spermatocyte.

6. The first spermatocyte chromosomes are formed in vesicles.

7. There are six dumb-bell-shaped bivalent chromosomes and one ovoid univalent chromosome in the primary spermatocyte.

8. The accessory is near the periphery of the nucleus and passes to one pole undivided slightly in advance of the other chromosomes in the first spermatocyte division.

9. The bivalent chromosomes divide crosswise.

10. The accessory chromosome divides in the second division.

11. The spermatozoön has an arrow-shaped head and a long filamentous tail.

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EXPLANATION OF PLATES.

All figures were made at table level by means of a Zeiss compensating ocular No. 6 and a 1.5 mm. objective with the aid of a camera lucida. The drawings were enlarged two diameters and then reduced one third.

PLATE I.

FIG. 1. Early prophase of spermatogonial division. *A*, a mass of chromatin which does not become reticular but remains more or less compact.

FIG. 2. Formation of spermatogonial chromosomes.

FIGS. 3 TO 6. Metaphase plates of spermatogonial chromosomes.

FIG. 7. Metaphase, lateral view, spermatogonial division showing position of the chromosomes on the spindle.

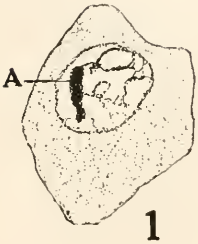
FIG. 8. Anaphase of spermatogonial division.

FIG. 9. Beginning of the growth period. *A*, a mass of chromatin which does not pass into a reticular condition and forms the accessory chromosome.

FIG. 10. Formation of chromatin thread.

FIG. 11. Synizesis.

FIG. 12. Beginning of the formation of the primary spermatocyte chromosomes.



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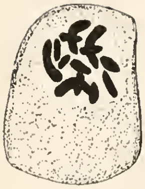

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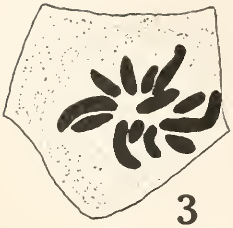

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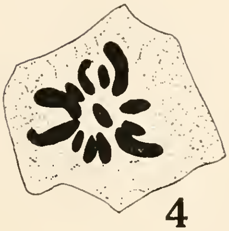

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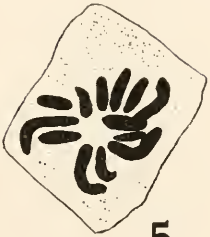

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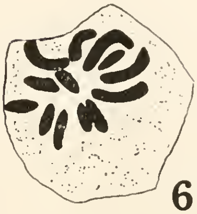

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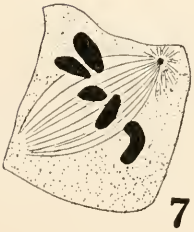

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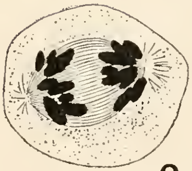

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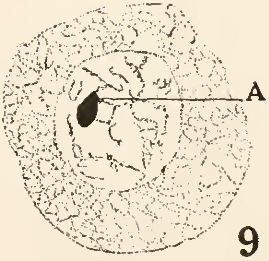

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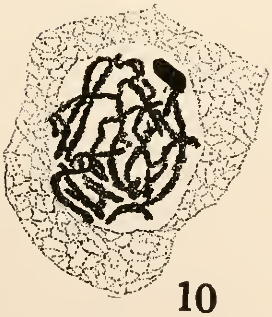

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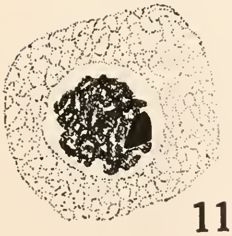

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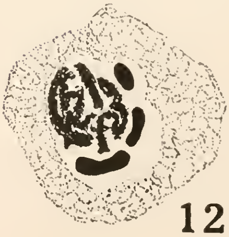

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PLATE II.

FIG. 13. Primary spermatocyte chromosomes showing the beginning of the formation of the vesicles.

FIG. 14. Primary spermatocyte chromosomes in vesicles.

FIGS. 15 AND 16. Metaphase, lateral view, of first spermatocyte division. The accessory chromosome is at the periphery of the spindle.

FIGS. 17 AND 19. Metaphase plates of first spermatocyte division showing seven chromosomes.

FIG. 18. Metaphase plate of first spermatocyte division showing six chromosomes. The accessory chromosome is not in the metaphase plate.

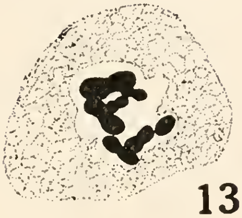
FIG. 20. Beginning anaphase of first spermatocyte division.

FIG. 21. Late anaphase of first spermatocyte division showing seven chromosomes.

FIG. 22. Metaphase, lateral view, of second spermatocyte division showing seven chromosomes.

FIG. 23. Metaphase plate of second spermatocyte division showing seven chromosomes.

FIG. 24. Metaphase plate of second spermatocyte division showing six chromosomes.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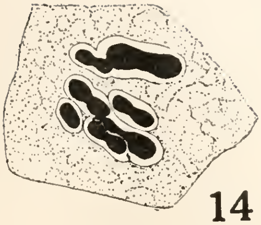
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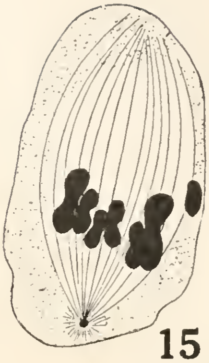
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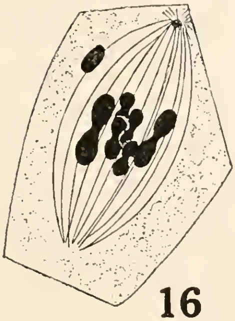
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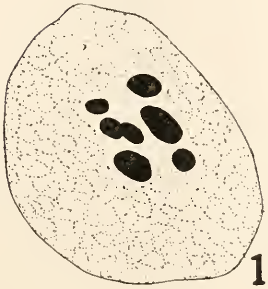
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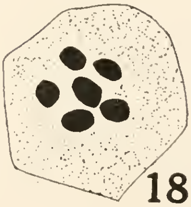
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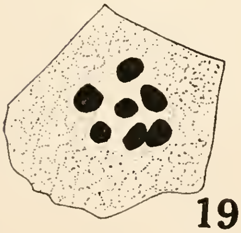
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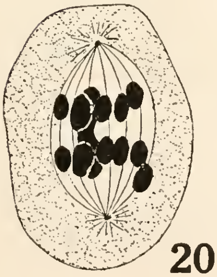
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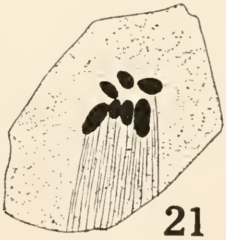
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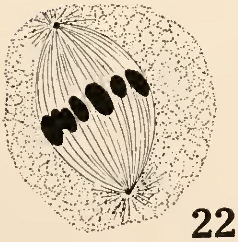
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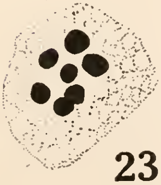
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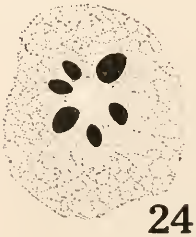
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PLATE III.

FIG. 25. Anaphase of second spermatocyte division showing seven chromosomes going to each pole.

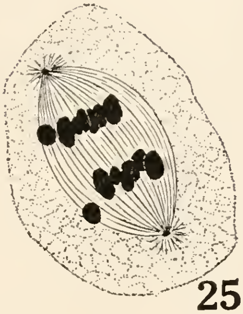
FIG. 26. Late anaphase of second spermatocyte division.

FIG. 27. Telophase of second spermatocyte division.

FIG. 28. Spermatids.

FIGS. 29 TO 31. Stages in the development of the spermatozoön.

FIG. 32. Head and part of the tail of a spermatozoön.



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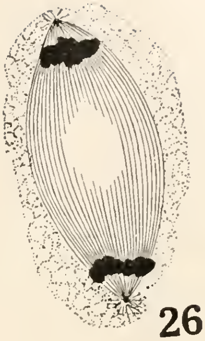

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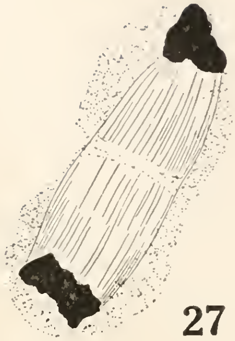

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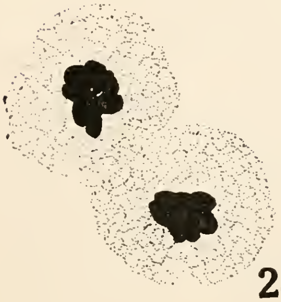

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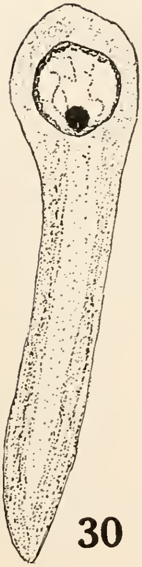

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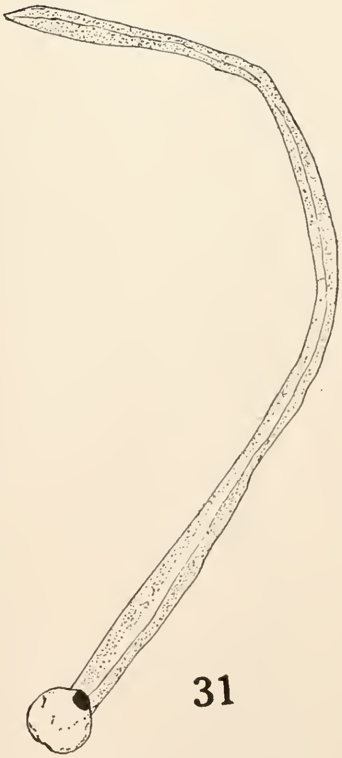

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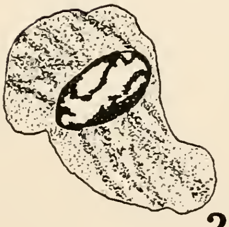

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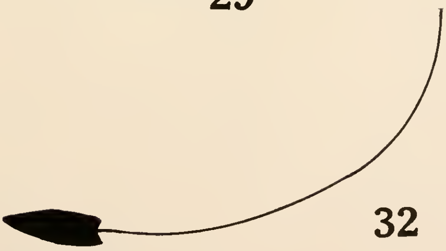

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MARY T. HARMAN

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BIOLOGICAL BULLETIN

NOTES ON THE BEHAVIOR OF THE ANT-LION WITH EMPHASIS ON THE FEEDING ACTIVITIES AND LETISIMULATION.

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INTRODUCTION.

The ant-lion is one of the marvels of the insect world and is discussed in practically every text-book on entomology and in almost every popular book on insects. With the exception of results derived from attempts to analyze the behavior of these insects into tropisms (4), European papers may be epitomized as follows: (1) the pits are formed in sand that is protected from the weather; (2) the larva excavates this pit by moving backward in a constantly narrowing spiral and using its abdomen as a plowshare and its head for a shovel; (3) with one of its forelegs, the ant-lion scrapes the sand on to its head from the inner side of the spiral; (4) with its body entirely concealed, the larva lies in ambush, with its open jaws resting in the bottom of the finished pit; (5) by tossing up sand at random, the ant-lion forces insects that alight on the side of the pit to tumble to the bottom; (6) any small terrestrial invertebrate may become its prey; (7) there is no mouth opening, the food being imbibed through tubes formed by each mandible and another mouth-part.

In American scientific journals, I have been able to find only four articles treating of our ant-lion. The first and the longest of these is by Emerton (6). In the fall of 1870, he found a pit of *Myrmeleon immaculatus* De Geer, under the shade of a boulder, at Danvers, Mass. The larva was carried home and placed in a bowl of sand. Immediately it buried itself. After remaining beneath the surface for several days, it excavated a pit. No mention is made of the larva using the foreleg to shovel sand on the flat head in the manner described by European and American popular writers. The larva was fed with flies. When given more than one at a time, it would kill all before eating any. It was kept over winter in a warm room. In May it spun its cocoon beneath the sand. In June the adult emerged, leaving half its pupa skin in the cocoon.

In August, 1871, Birge (2) found a colony of 600 ant-lions, under an overhanging cliff, in Albany County, N. Y. These pits were in a soil composed of fine disintegrated limestone commingled with pebbles and minute fragments of stone. Whenever an insect alighted upon the sides of the pit the ant-lion began to toss up the soil in all directions.

Moody (14) states that the ant-lion observed by him rests at the bottom of the pit with its jaws only showing, and that it throws up sand at escaping prey. It formed its cocoon June 4 and emerged July 8.

Moffat (13) writes: "Fine loose sand is evidently a necessity of their existence in any locality." He mentions the throwing up of sand when an ant steps on the side of the pit.

The Pit.

Most accounts give the erroneous impression that the pits of ant-lions are formed only in sand. Even in a scientific magazine, Moffat (13) writes, "Fine loose sand is evidently a necessity of their existence in any locality." A loose friable soil protected, more or less, from rain and shielded from chickens and similar insect feeders is all that is needed. It may be anything from the finest dust to coarse sand. In open sheds with dirt floors, under porches where the place is not too dark, beneath low railroad bridges that span sandy, dusty, or cindery ground, under ledges of rock, and beneath the shelter of logs that do not touch the ground at all points are good places to look for them. From time to time, during the past three years, I have had, in my insectary, more than 500 ant-lions. Many of these were obtained in Kansas by my friend, Mr. Phil Rau; the remainder were collected in and about St. Louis. The majority of these were found in the loamy clay (loess) that forms much of the top soil of Missouri and Kansas; some were found in cinders in sheltered places along railroad tracks; some, in disintegrated mortar along the walls of buildings; some, in the rotton-wood dust of hollow logs; none of these was obtained from sand. In the wide jelly glasses of my insectary, where each larva was kept in solitary confinement, some were placed in loamy clay, some in sifted coal ashes, some in coal ashes that had been weathered for a year, some in fine sand and others in coarse. The larvæ seemed to flourish as well in one medium as in the other.

The ant-lion usually begins the construction of its pit by striking out a circle in the friable earth. Using its abdomen as a plowshare and its head as a shovel, the larva burrows backward, in a circular path, just beneath the surface of the soil, tossing upward and outward the dirt that falls upon its head. Almost all of the articles that I have read state that this initial circle marks the outer boundary of the finished pit. With the American ant-lion of the Middle West this is not always so. In most cases observed by me the finished pit is wider than the diameter of this circle. In the first place, the falling inward of the soil as the excavation progresses enlarges the diameter. Then, too, the ant-lion sometimes enlarges the partly or apparently entirely completed pit. After this first circle is completed, within this the ant constructs, in a similar manner, a deeper adjacent circle and so on until the center is reached. Then, with the major portion of its body hidden in the walls of the pit and using its head and mandibles as a shovel, it tosses out the material from the bottom of the pit, until the dirt no longer runs down the sides.

European writers state that the ant-lion shovels sand on to its head by means of one of its forefeet, and Kirby and Spence (8) insist that, in excavating its burrow, the ant-lion reverses the direction it is going at the completion of each circle, so as to alternately exercise each foreleg. In our American ant-lion this pair of forelegs functions, not as a scrape, but as a brace to the body when the ant-lion is shovelling dirt or turning. Patient watching with a magnifying glass has failed to detect the forefoot loading dirt upon the head; and certainly the ant-lion does not reverse the direction it is going every time it completes a circle. The dirt gets upon the head by falling from above and from the sides, as the larva burrows backward through the soil. Some of this material comes from the outer edge as well as from the inner. While constructing its pit, the larva often pauses. After each rest it usually continues in the direction that it was going. On rare occasions, it does turn about and go in the opposite direction. This is usually when it has met some obstruction.

To test the matter more thoroughly, a sufficient portion of each of the forelegs of an ant-lion was amputated to render them much too short to be of value in shovelling soil on to the head. As soon as it was returned to its glass, the larva burrowed backward into the soil. For four days it remained beneath the surface. On that day it excavated a small pit. The next day the pit had been enlarged. On this day it was fed with ants (*Formica subsericea*). The ant-lion was then removed from the soil and examined under a simple microscope. The legs had not regenerated; each stump was covered with a ball of soil. This ant-lion had constructed its burrow without using its front feet as scrapers with which to load dirt on the head.

The force with which an ant-lion tosses the materials from its pit is astonishing. Often they are cast several inches beyond the rim. Sometimes the larva encounters particles which cannot be disposed of with a toss of the head. When these are not too heavy the insect has an unique method of disposing of them. The insect backs up the side of the pit with the obstacle poised on the posterior portion of its abdomen and deposits it beyond the edge of the pit. Although this behavior is described by Bingley (19), most writers do not mention it. Perhaps it sounds too much like a fairy tale; yet it is comparatively easy to induce an ant-lion to behave in this manner. I frequently induced it either by placing a small stone in the center of the ring of a pit that was being constructed; or, by depositing a similar object in the bottom of a completed pit. When the stone is placed in the center of the ring, as the ant burrows spirally inward, there is sure to come a time when the stone will fall into the furrow. When the ant-lion returns to that point it encounters the obstacle. Usually it burrows under the object and continues on part of the way around the circle. Then, turning, it backs through the furrow thus made until it has inserted the tip of its abdomen under the impediment. It then backs slowly up the slope with the burden poised upon the tip of its abdomen. The edges of the abdominal somites and the stiff bristles thereon prevent the stone from slipping forward; while the dirt on each side prevents it from falling sidewise. Throughout this entire upward journey the whole body of the ant-lion is above the ground. It is an astonishing sight to see the insect backing, in almost a straight line, up the steep slope, with the burden poised on its back. When the burden has been disposed of, usually at the edge of the pit, the ant-lion turns about and returns to the bottom of the pit, usually in the furrow made by the upward struggle, and continues her digging. The furrows made before my eyes have always been straight or nearly so; but, one made in my absence was quite curved. When the object was placed in the bottom of a finished pit, sometimes the object was allowed to remain; but, in most cases, sooner or later, it would be removed, in the follow-

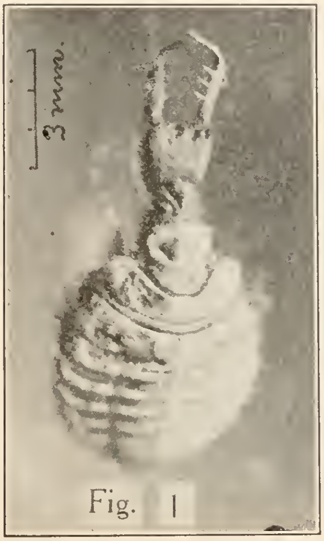


Fig. 1

Larval ant-lion. Dorsal view.

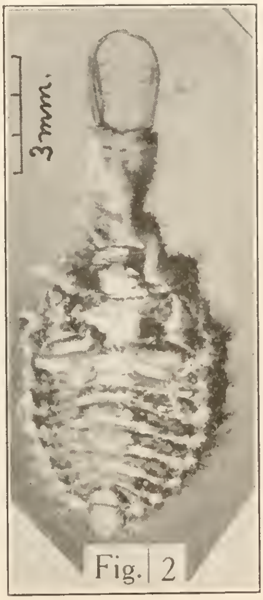


Fig. 2

Larval ant-lion. Ventral view.

ing manner. When it had tossed up a few loads of dirt, the larva would back away from the obstacle in a straight or a curved line; then turning, it would back through the furrow thus made and proceed as described above. When the stone is too heavy for the insect to handle in the manner mentioned above, it either deepens the pit on one side of the obstacle, or buries the obstacle by mining under it, or else abandons the pit. In several important respects the behavior observed by me differs from that described by Bingley; (1) never once did I see the stone fall from the insect's back and roll to the bottom of the pit, (2) obstacles encountered in constructing the pit were usually removed at once, (3) such bodies were usually deposited just beyond the rim of the pit, (4) occasionally they were left on the side of the pit.

On rare occasions I have seen pits constructed in a different manner. Instead of beginning by striking out a circle, the ant-lion burrowed downward into the ground and began at once casting out the soil, thus making a pit of small diameter. Usually such pits were afterwards enlarged by burrowing into the walls and proceeding about as described above.

Thus my experience with the pit-building behavior of the ant-lion harmonizes with McCook's account (10); but is not in accord with that of Mrs. Comstock (5) who writes: "Wonderful stories are told about the way ant-lions dig their pits, marking out the outer margin in a circle and working inward. However, our common ant-lion of the East simply digs down into the sand and flips the sand out until it makes a pit."

The magnitude of the pit and the slope of the sides depend upon the size of the larva and the nature of the soil; the coarser the individual particles and the greater their specific gravity the more gentle the slope. In the loess about here the pits vary in diameter from less than half an inch to about two inches; the latter being the more abundant. Often the depth of the pit is almost as great as the diameter. Although a small ant-lion usually excavates a small pit, a small pit does not necessarily contain a small larva; for large larvæ sometimes construct small pits which they afterwards enlarge.

Occasionally one finds an isolated ant-lion pit; usually they occur in groups (Figs. 10, 11). In the same cluster the ant-lions differ much in size, and this is true even in the early spring. Certain writers attribute these differences in size to the fact that some obtain more food than others. The following simple experiment lends support to this view. From a certain well-circumscribed area, containing about fifty ant-lion pits, a dozen larvæ were removed, on June 22, and placed in my insectary. A portion of these were well fed daily, the remainder were fed only occasionally. A few were lost by accidents. By August 8 all of the survivors of the well-fed lot had formed cocoons and a few imagoes had emerged. The poorly fed individuals were still larvæ. The majority of those left in the field were still larvæ.

Morphologically the ant-lion (Figs. 1–3) is well adapted to this pit-building behavior. The flat head, which, with the stout mandibles, forms an excellent shovel, is so articulated to the rest of the body that it is possible to give it a powerful upward jerk. The abdomen is flat on the ventral and convex on the dorsal side, the whole tapering toward the tip in such a manner as to form an excellent burrowing instrument. From the sides of the body clusters of stiff bristles project outward and forward in such a manner that the body is prevented from slipping forward after it once has penetrated the earth. Then, too, the terminal claws on the legs (Fig. 12) make efficient anchors. The front of the dorsal portion of the prothorax is so rounded that dirt easily

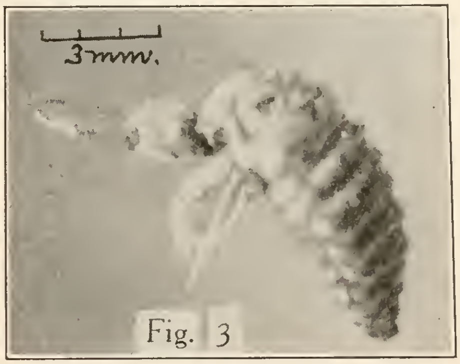


Fig. 3

Larval ant-lion. Lateral view.

falls forward and loads the shovel-like head. There is no functional anal opening; hence there is no danger of vigorous thrusts of the abdomen clogging the intestine with dirt.

Feeding Behavior.

The finished pit is an inverted hollow cone, at the apex of which the wide-open mandibles of the larva, with their sharp teeth, await to grasp any unfortunate that happens to fall therein. What an efficient trap for small creeping invertebrates! The steep and unstable sides often cause the animals to fall to the bottom. If the intruder does not at once slide to the bottom, its struggles to escape tumble the soil upon the mandibles of the waiting ant-lion. Immediately the ant-lion begins to toss the soil upwards. The claim that the dirt is cast at the struggling creature is erroneous. Digging its mandibles edgewise into the bottom of the side of the pit, the ant-lion shovels out head-load after head-load of soil. It is not thrown at something; it is simply tossed upward and outward. Some of these random shots may take effect; and the constant undermining of the walls of the pit produces miniature landslides which, usually, drag the prey to the bottom of the trap.

Until something falls into the pit or alights on its treacherous sides, these mandibles of the larva usually rest horizontally in line with the body, which is hidden in the wall of the pit. Ordinarily the pits appear to be empty, for the mandibles are often covered with fine dirt. Even when the whole head is uncovered, its color harmonizes so perfectly with that of the soil as to render it invisible. As soon as its jaws close upon a creature the ant-lion backs deeper into the walls of the pit and, by interring its victim, subdues it. Thus the ant-lion is enabled to conquer creatures that are much larger and apparently stronger than it. Unless its first few struggles free it, the captive is doomed; for the ant-lion slowly but surely drags it deeper and deeper into the soil, while it feasts on its body juices. To assist in holding the prey while its body contents are being imbibed through the hollow mandibles, each mandible is provided, on its inner surface, with three stout teeth (Fig. 13*A*).

MacLachlan (11), in discussing the feeding of the European ant-lion (*Myrmeleon formicarius*) says: "The house flies and other small insects were usually dragged partially or wholly under the sand, whilst blue-bottles and similar bulky creatures were feasted upon on the surface." In his account of the feeding of the common ant-lion of the east, McCook remarks: "The ants were held off at 'arm's-length,' so to speak, and were thrashed and jerked about until they were exhausted. Meanwhile efforts at defence were made futile by the captor, who held its victim out of reach of any vital part." Neither of these accounts tallies exactly with my experience, although MacLachlan's can be harmonized with it. I have watched ant-lions feed thousands of times and have fed them with a variety of invertebrates. In every case the larva has attempted to drag its captive beneath the ground. In no case was the insect held off at arm's length as described by McCook. Often, a few moments after its capture, all that would be visible of an ant were the tips of its waving antennæ, or the extremity of its wriggling abdomen, or both. Naturally the captive struggled and squirmed; but there was no attempt on the part of the ant-lion to hold its prey at arm's length above the ground, while it thrashed it and jerked it. If the first closing of the mandibles does not capture the creature that

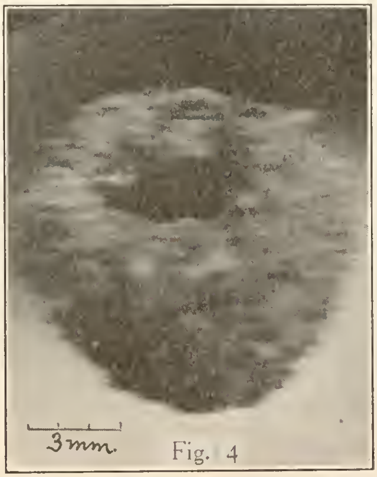


Fig. 4

Empty cocoon of ant-lion.

happens to fall into the pit, remaining at its post, the ant-lion elevates its head and makes repeated snaps at the creature as long as it remains near. It may be that the ocelli located at the base of the mandibles, on the dorsal side of the head, aid in this.

The name ant-lion is a misnomer; for it creates the impression that this insect feeds exclusively, or almost exclusively, upon ants. Such is not the case. Any small creeping invertebrate—be it insect, crustacean, or arachnid—is acceptable. Several of the most flourishing colonies of ant-lions found near St. Louis are located in the dirt floor of a dilapidated stone-crusher of an abandoned quarry. The diet of the inmates of those pits is composed largely of sow-bugs (*Porcellio*). Emerton (6) and MacLachlan (11) fed their ant-lions on living flies that had been disabled; Berce (1) reared his on living flies, wood-lice and earwigs. I supplied mine with living specimens of the following invertebrates; caterpillars (even hairy ones), wood-lice, small roaches, small moths (held by the wings until the ant-lion had secured a hold), spiders, nymphal squash bugs, ants, small beetle larvæ, soft-bodied beetles, and bed-bugs. All of these were accepted and, after the juice had been sucked from each body, the dried remains were cast out of the pit.

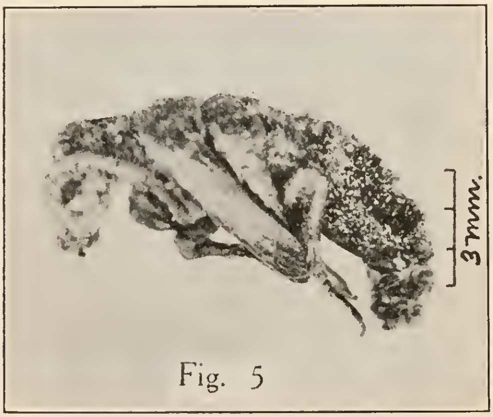


Fig. 5. Chrysalis of ant-lion that died on way to surface.

The ant-lion has no mouth opening in the true sense of the word. The strong curved mandibles are perforated at the tip, and along the ventral surface of each there runs a prominent tube through which the juices of the victim are sucked. This tube is composed of two parts. Along the whole of the ventral surface of each mandible [Fig. 13*A*] there is a deep groove with incurved edges. Another mouth part [Fig. 13*B*], probably the maxilla, fits so tightly into this groove of the mandible that, even when viewed with a 2/3-inch objective, the two seem to form a single structure. With that power, on the underside of each mandible one sees two ridges. These mark the junctions of the two pieces; but, unless you had previously dissected a mandible, you would not suspect that there were two pieces and that they were not rigidly united. Turn the insect on its ventral surface, carefully disarticulate the mandible, and, with a pair of dissecting needles, gently push it forward. Thus the other mouth part will be gradually drawn out of the mandible and left attached to the ventral part of the front of the head.

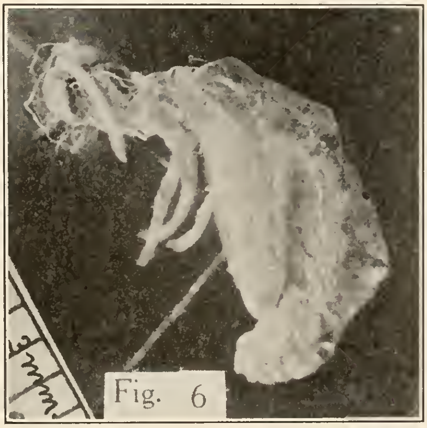


Fig. 6

FIG. 6. Shed chrysalis skin of ant-lion.

The ant-lion preys upon living invertebrates. How does it distinguish the living from the not-living? There may be several factors which help it solve this problem. The following experiments show that one attribute by means of which the ant-lion differentiates between desirable and undesirable prey is the exhibition of restlessness:

Experiment 1.—I fastened a bit of straw to the end of a silk thread.

Twirling the other end of the thread between my thumb and fore-finger, I gently lowered it to the bottom of the pit. Three times in succession the ant-lion caught hold of the straw with its mandibles. Each time I jerked the string and thus removed the straw from its grasp.

Experiment 2.—I fastened a dead chinch bug to one end of a silk thread. Twirling the other end of the thread between my thumb and fore-finger, I gently lowered the bug to the bottom of the pit. Immediately the ant-lion seized it with its mandibles and held on until, by pulling on the thread, I had drawn the insect partly out of the pit. This experiment was tried with five different individuals. Four responded in the manner just described; the fifth made no response.

Experiment 3.—I fastened a piece of cotton to one end of a silk thread. Twirling the other end between my thumb and fore-finger, I gently lowered it to the bottom of the pit. The ant-lion gripped the bit of cotton with its mandibles and held on until I, by pulling on the thread, had dragged the larva partly out of the pit. This experiment was tried with five different individuals. The result was always the same.

MacLachlan (11), in 1864, placed between two and three dozen ant-lions in a small box of sand and carried them from Fontainbleu, France, to London. When he arrived, about half of the larvæ had been killed and the juices of their bodies extracted by the others. In shipping ant-lions to me from Kansas, Mr. Rau placed a hundred or more in the same small box of dirt. In sorting over the material to place each one in an individual retainer, I always found several dead specimens that looked as though the juices of their bodies had been extracted. Are these deaths caused by cannibalism? To test the matter, an ant-lion was dropped into the pit of another individual. This experiment was repeated over and over again throughout a summer devoted largely to the field study of this creature. In the majority of cases the intruder escaped either by burrowing into the wall of the pit or else by backing out of it. In several instances, however, it became the prey of the rightful owner of the pit. Evidently, when opportunity permits, this creature is a cannibal.

LOCOMOTION.

The forms of locomotion used in excavating pits and in removing obstacles therefrom have been described in the section on "The Pit" and will not be repeated here.

When placed on loose dry soil, the ant-lion may letisimulate. As soon as it begins to move, it burrows backward into the ground. If an ant-lion is placed in an open rectangular pasteboard box, it backs along, sometimes in a straight line and sometimes in a curved line, until it comes in contact with one of the sides. It then backs along that side until it comes to a corner, turning the corner it continues along to the next corner. It may continue thus for a long time, or it may vary it by creeping backwards up one of the angles until it reaches the top of the box and then pass downward to the ground. After it has once reached the side of the box, no matter how long it remains within the box, it almost never moves out into the open. These two simple experiments indicate that this insect is positively thigmotactic. With this statement must be coupled the reservation that, at times, the creature moves about with all of the upper portion of the body

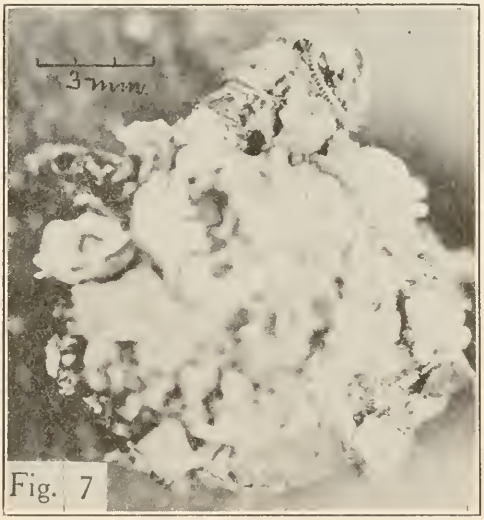


FIG. 7. Cocoon of ant-lion, with chrysalis partly emerged. This cocoon is from a form that was raised in shifted coal ashes.

exposed. This is the case when it is removing an obstacle from its pit.

Since this larva burrows downward into the earth, it may be considered positively geotactic; but, it must be remembered that it does not always pass downward. When disturbed in its pit, it usually backs upward, just beneath the surface, until the rim is reached; sometimes, it continues onward, in a horizontal direction, beneath the surface. MacLachlan (11) observed that, at night, they frequently make perigrinations over the surface of the ground. Then, too, they sometimes ascend vertical surfaces.

When placed on a horizontal surface [I used sheets of brass, glass, wood, and cardboard], the larva backs away from the light. If placed with the tip of its abdomen toward the source of light, usually, it will move a short distance toward the light then turn, to either the right or the left, and back away in a straight line. This, coupled with the fact that, when placed on its back, the ant-lion invariably rights itself by turning away from the source of light, induces the conclusion that this creature is negatively phototactic; but, it must be remembered that in

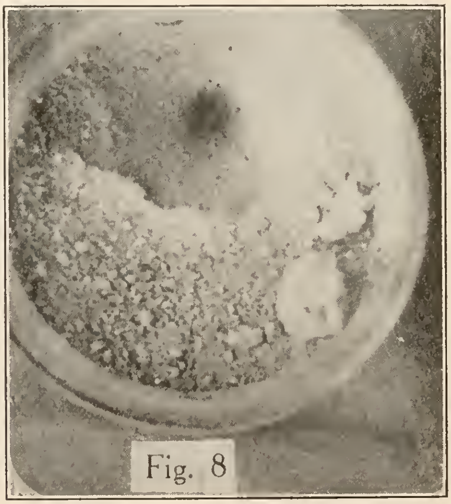
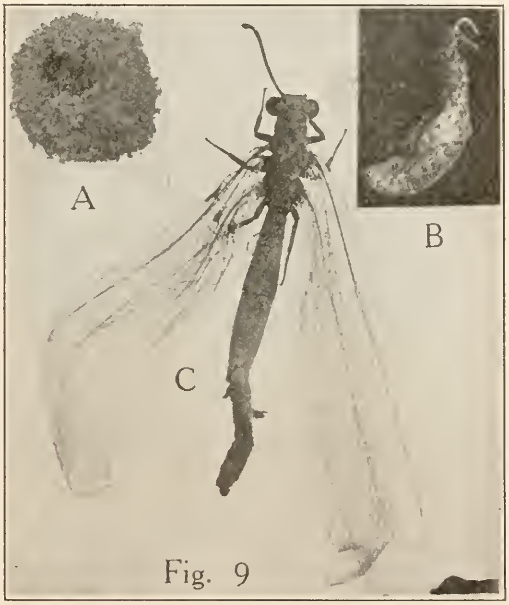


Fig. 8

Ant-lion pit in one of my tumblers.

constructing its burrow the larva crosses the light at every possible angle, and that, at times, it moves toward the light.

This insect invariably moves backward; never under any conditions does it move forward; yet it is capable of performing all the ambulatory feats possible to an insect that progresses in the orthodox way. It can move in a straight line, it can describe simple or s-shaped curves to either the right or the left, and it can ascend or descend rough surfaces inclined at any angle from zero to ninety degrees. By means of what structures does it perform these movements? Are they produced entirely by flexing the abdomen and trusting to the body bristles to prevent forward movements? Do the legs take any part in the movements? Do the mandibles assist? To answer these questions the following experiments were devised.



Photograph showing the comparative size of cocoon, chrysalis and imago. They belong to the same ant-lion and are photographed to the same scale. *A*, cocoon; *B*, chrysalis skin; *C*, imago.

Experiment 1.—An ant-lion was placed on a glass plate arranged horizontally. By means of a hand magnifying glass every movement *was watched.* It moved backward in jerks. The hind legs, which were doubled back under its abdomen, made jerky pulls. The middle pair of legs was directed outwards in almost a straight line. The anterior pair of legs was stretched forward. The tips of both the first and second pair of legs touched the glass. The mandibles took no part in the movement.

Experiment 2.—The ant-lion was placed on the glass plate and held, in a horizontal position, above my head; so that I could look up at it with a magnifying glass. The results were the same as in experiment 1; but it was easier to observe that the tips of all the legs touched the glass. The third pair of legs was the only pair making vigorous movements.

Experiment 3.—I tilted the glass plate so that the posterior portion of the ant-lion was uphill. When the angle became steep the ant-lion fell.

Experiment 4.—Repeated number 1, substituting a pasteboard rectangle for the glass plate. The result was the same as in experiment 1; but the insect moved faster.

Experiment 5.—I tilted the paste-board rectangle so as to have the posterior portion of the insect up-hill. Even when it had reached an angle of 90 degrees, the insect retained its hold. It moved upward, sidewise and downward.

Experiment 6.—While the cardboard rectangle was inclined at a steep angle and the ant-lion was resting head downward, with a dissecting needle, I raised the tip of the abdomen from the support. The ant-lion retained its hold.

These experiments show conclusively that the mandibles do not assist in locomotion; at the same time, they indicate that the hind pair of legs play an important rôle. Yet, so far as these experiments go, the hind legs might be mere grappling hooks to prevent the creature from slipping forward and the real locomotion be due entirely to the flexing and stretching of the abdomen, all forward motion being prevented by the stiff bristles on the sides of the body and the grip of the legs.

Experiment 7.—A layer of dirt equal to the height of the greatest height of the ant-lion was spread on a glass plate. The ant-lion was placed on this pile of dirt. The larva began to burrow backward into the dirt; but made practically no progress. By the behavior of the body I could see that it was making vigorous movements with its third pair of legs; but it made practically no progress.

Experiment 8.—A layer of dirt equal in elevation to the greatest height of the ant-lion was placed on a pasteboard rectangle. An ant-lion was placed on this pile of dirt. Immediately it began to burrow backward and continued to progress at a rapid rate.

(In all of the experiments from 1–8 the same individual was used. The series was repeated many times with different individuals; but, in each case, the same individual was put through the eight experiments.)

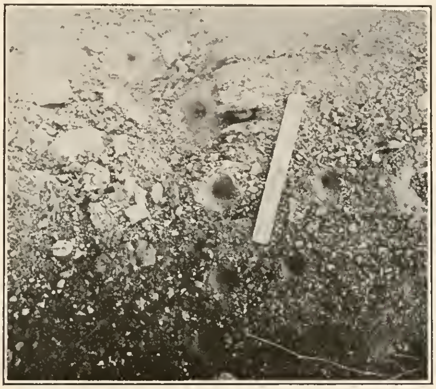


FIG. 10. A cluster of ant-lion pits (average cluster).

In experiments 7 and 8 the presence of the dirt makes it necessary for more work to be performed in making progress backward. Since the height of the pile is the same in each case, the amount of work required is the same. Since the bristles are more numerous on the sides of the body than on the ventral surface, the presence of the dirt should give an added opportunity for them to function in preventing forward slipping of the body; hence, if the progress is due simply to a flexing and stretching of the body the ant-lion should be able to move just as fast, if not faster, on a glass plate with a layer of soil as on the naked glass. If, however, the hind legs play an important rôle in dragging the body backward, then the larva on the dirt-covered pasteboard should have a great advantage over the one on the dirt-covered glass plate. These experiments prove, I think, that the hind legs assist in dragging the body backward. A microscopic examination of the legs reveals two terminal claws which function in this work (Fig. 12).

EMERGENCE OF THE IMAGO.

This section does not pretend to be a life history of the ant-lion. That the author hopes to make the subject of a future paper. This is simply an attempt to state some interesting facts about the last stages of the metamorphosis.

At the close of its larval period, the ant-lion constructs a subterranean spherical cocoon of silk and soil. In my insectary, most of the cocoons have been formed quite near the surface; sometimes projecting slightly above the soil. In one case, however, I found a cocoon on the bottom of the jelly glass, fully two inches below the surface. In my insectary, the cocoons have been formed in July and in August and the imagoes have emerged in from 9–20 days thereafter; but I do not consider that I have sufficient data to warrant the statement that they are always formed at those times.

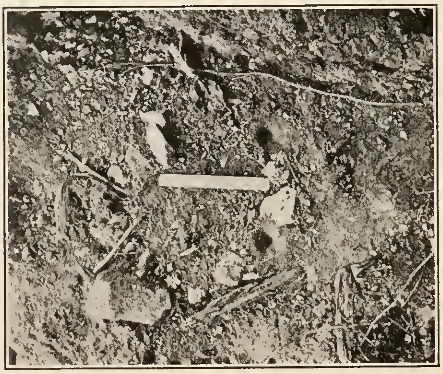


FIG. 11. A cluster of ant-lion pits (small cluster).

Inside of this cocoon the insect sheds its last larval skin and becomes a chrysalis. At the end of a certain period of time the anterior portion of the thorax protrudes from the cocoon (Fig. 7).

All of the accounts that I have read state that the chrysalis comes about half way out of the cocoon and from its dorsal surface the imago emerges. In my limited experience I have noticed three methods of emergence. In one case the chrysalis protruded about half way out of the cocoon and the imago emerged from its back. In another case the chrysalis had left the cocoon entirely and protruded about half way out of the soil. In the third case both the head end and the tail end of the chrysalis remained within the cocoon and from its back the imago emerged. I am inclined to think the third case abnormal, caused by the head of the chrysalis becoming entangled in some strands of the cocoon. Fig. 6 is a photograph of the cast skin of that chrysalis, made just after I had removed it from its cocoon. It seems to me that the other two cases may be explained as follows: when the cocoon is near enough to the surface for the chrysalis to expose the upper portion of its body without coming entirely out of the cocoon it does so; when the cocoon is a little deeper then the chrysalis leaves the cocoon entirely and continues upward until the anterior portion of its body is above the surface.

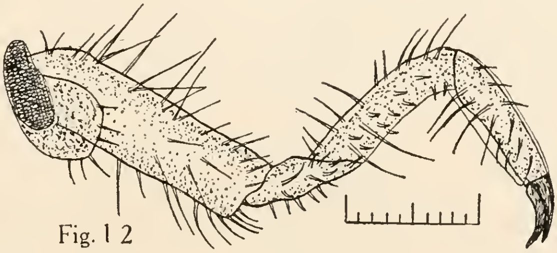


FIG. 12. One of the third pair of legs of an ant-lion larva.

When the cocoon is too far beneath the surface, the chrysalis dies on its upward journey. Fig. 5 is the photograph of such a chrysalis. It was found dead about half an inch below the surface. Attached to the bottom of the jelly-glass—about an inch below—the empty cocoon was found.

Soon after emerging the imago undergoes an enormous increase in size. It soon becomes more than twice as large as the chrysalis from which it came, and this without partaking of food. Fig. 9 illustrates this. The jelly glass containing the cocoon had been tightly closed to prevent the possible escape of the imago when it emerged. It emerged at an unexpected time and when discovered it was dead. It had lost one antenna and its body was slightly damaged. Under the conditions it could not possibly have obtained food. Half exposed above the soil was the shed chrysalis skin, and a short distance below the surface the empty cocoon (Fig. 4) was found.

Miscellaneous Activities.

Experiment 1.—A ring of water eight inches in diameter was made on a glass plate and an ant-lion placed in the center of the dry space that the ring surrounded. The ring was one half of an inch in width. When the ant-lion reached the ring of water, it would usually turn and move away from it. Often, in turning, its mandibles would get into the water. In that case the mandibles would leave a broad water band wherever the creature went. After its mandibles had become wet, on its next approach to the water, it was apt to get some other part of its body wet. After that it was apt to move, away from the light, on through the water.

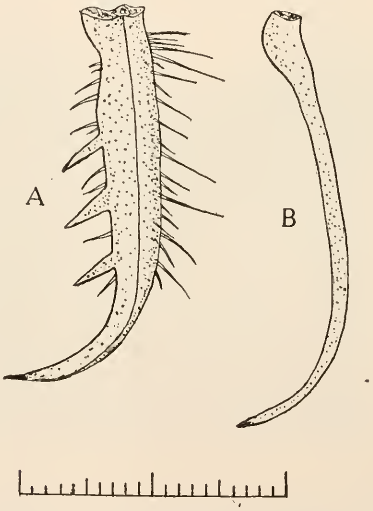


Fig. 13. The parts that form the sucking tube of the ant-lion larva. *A*, mandible. *B*, the part that fits into the groove of the mandible.

Experiment 2.—To see if there was anything about dirt as such that would direct an ant-lion to it, a pile of dirt three inches in diameter was placed in the center of a glass plate that was twelve inches *square. The ant-lion was placed at various places near the periphery of the plate.* Unless the ant-lion was placed in such a position that in going directly away from the light it would encounter the dirt, never once did an ant-lion discover it. Sometimes the larva passed within less than a centimeter of the dirt without being attracted by it.

LETISIMULATION.

Letisimulation (from *letum*, death, and *simulare*, to feign) is a term coined by Weir[1] in 1899, to designate the death-like attitude assumed by individuals of many different groups of the animal kingdom, when roughly handled. While citing examples from among the worms, insects, reptiles, birds, and certain mammals, he leaves the impression that the most remarkable examples of death-feigning are to be found among the reptiles and certain mammals. Since that time much careful attention has been given to the letisimulation of insects. Barret (22) has studied it in the mole-cricket; Gee and Lathrop (26), and Johnson and Girault (32), in the plum curculio; Girault (27), in trox; Holmes (30), in the water scorpions; Newell (34) and Weiss (40), in the rice weevil; Riley (36), in dragon-fly nymphs; the Severins (37), in the giant water bugs, and Wodsedalek (41, 42), in May-fly nymphs and in a dermestid larva. In the light of the remarkable traits revealed by these investigators, were he writing his article today, Weir, no doubt, would agree with Homes that "it is among the insects that the death-feigning instinct reaches its highest development, occurring in a greater or less extent, in most of the orders. It is especially common in beetles and not unusual among bugs, but it is quite rare in the highest orders such as the Diptera, or flies, and the Hymenoptera, or ants, bees and their allies. It occurs in a few cases among the butterflies and moths, both in the imago as well as the larval state. The instinct is exhibited in different species in all stages of development from a momentary feint to the condition of intense vigor lasting for over an hour. Some insects may be severely mutilated, or, according to De Geer, even roasted over a fire before they cease feigning."

Although the activities of ant-lions have interested many naturalists, very little attention has been paid to their death-feigning behavior. Emerton (6) asserts that rough handling caused his specimens to remain inactive for a time, and MacLachlan (11) states that the form observed by him letisimulates. Each of these students dismissed the matter with a single sentence.

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[1] Weir, "Dawn of Reason," pp. 202–214.

The results recorded in this article are based on a careful laboratory study of 100 individuals selected at random; supplemented by observations made in the field. About 60 per cent. of these came from Kansas; the remainder from the vicinity of St. Louis, Mo. Some were quite small and others were almost large enough to form their cocoons. They were isolated in numbered jelly-glasses, partly filled with loamy loess, and were kept in an out-of-doors insectary, the north wall of which was constructed of wire netting. The other walls were opaque.

Any stimulus which produces a shock will usually cause an ant-lion to letisimulate. Rough handling, roughly turning it upon its back, dropping it from a slight elevation, all have a similar effect. I usually induced the feint by roughly turning the larva upon its back, or by dropping it from a slight elevation. Occasionally I found an individual that I could not induce to letisimulate; but this was a very rare occurrence.

Several investigators have thought it important to determine if the poses of letisimulating individuals are death attitudes. Based on a consideration of seventeen species of invertebrates, Holmes (31) concludes that the poses assumed were usually quite different from death attitudes; although there were some species in which they were always identical. I find that the ant-lion has not one, but several death attitudes; likewise it possesses a number of death-feigning postures, some of which resemble a death pose and some of which do not. The insect suddenly becomes rigidly immobile in whatever attitude it may be when it receives the shock. Absolute immobility is the character that is common to all cases; when the feint follows a long period of fasting, this inactivity often simulates death. The rigidity, however, is not so great as that described for certain insects. In some species of insects the rigidity of the parts during a death feint is so great that the insect may be picked up by a tarsus and held out at right-angles without the leg bending in the least. That is not the case with the ant-lion. When an attempt is made to lift it, in that manner, by a tarsus, the leg bends and the insect awakes from its feint.

When an ant-lion is recovering from a feint, usually, although not always, there is a preliminary waving of the antennæ and a twitching of the legs and, sometimes, a movement of the head. Then the larva suddenly turns over. Throughout the whole series of experiments, a careful record was kept as to whether the insect turned towards the right or towards the left; towards the light or away from it. It was found that whether the insect turned toward the right or toward the left depended upon the location of the strongest light; for the ant-lion invariably turns away from the light.

Pinching the legs of a letisimulating individual almost always caused it to come out of its feint. Blowing upon a death-feigning larva would sometimes bring no response; at others it would induce a twitching of the legs; at yet others it would cause a complete recovery. Since the pinching of a leg, and even attempts to lift the letisimulating ant-lion by a leg, usually terminates the feint, I was surprised at the results produced by the following mutilations.

Experiment 1.—With a pair of small, but sharp, dissecting scissors, I cut off the tip of a fore-leg of a letisimulating ant-lion. The insect did not respond.

Experiment 2.—With a pair of small, but sharp, dissecting scissors, I clipped off the tip of a mandible of a letisimulating ant-lion. The insect did not respond.

Experiment 3.—With a pair of small, but sharp, dissecting scissors, in rapid succession, I cut off the tips of both fore-legs and of a mandible of a letisimulating ant-lion. The insect did not respond.

Experiment 4.—With a pair of small, but sharp, scissors I severed the head from the body of a letisimulating ant-lion. The insect did not respond, nor did it recover from the operation.

Experiment 5.—A pair of small dissecting scissors, identical with those with which the above experiments were performed, was heated red hot, in a Bunsen flame, and allowed to cool. This softened the *steel and made the edges quite dull. With these scissors an attempt was made to remove the tip of a leg of a letisimulating ant-lion.* The scissors were too dull to cut through the chitin; instead of being severed, the leg became wedged in between the blades. The feint was terminated immediately.

With the exception of experiment number four, these amputations were performed several times. On one occasion, a larva recovered the moment I cut a leg; on another day, the same thing happened when I severed a mandible. With these two exceptions, the results were always as stated above. How shall we harmonize the fact that the pinching of a leg or, sometimes, the blowing of the breath on the larva terminates the feint, while the severing of a leg, or a mandible, or both invokes no response? Shall we decide that such a pinch produces a greater physiological shock than a sudden cut with a pair of sharp scissors? Is it possible that a breath of air produces a greater shock than an amputation with sharp instruments?

Relative Duration of Successive Death Feints.—In his study of the beetle *Scarites gigas* Fabre (24) found that the duration of the first five successive feints gradually increased from the first to the last. The Severins (37), in their study of the giant water bugs *Belostoma* and *Nepa*, and Gee and Lathrop (26), in their study of the plum curculio (*Conotrachelus nemuphar*), find great irregularity in the lengths of the successive feints.

To test the matter, an ant-lion was removed from its pit, placed on a board, and made to letisimulate by roughly turning it on its back. As soon as it recovered from one feint, it was roughly turned on its back and induced to letisimulate again. This was repeated until it had had an opportunity to letisimulate twenty times. By means of a stop-watch, the duration of each feint was obtained. One hundred individuals were thus experimented with and the results recorded in a table. Criticially examined, the table revealed a number of interesting things. (1) There are marked individual variations. (2) In twenty opportunities the individual usually letisimulates less than twenty times. (3) The total time consumed in twenty opportunities to letisimulate varied from one minute to two hours and twenty-three minutes. The average for the 100 individuals was nineteen and six-tenth minutes. (4) This death-feigning cannot be indefinitely prolonged. (5) The duration of the feints near the end of a long series of trials is always shorter than that of the earlier ones. (6) A curve representing the relative lengths of a series of letisimulations always contains two or more crests. (7) The longest feints usually occur somewhere near the beginning of the series. Of the 100 cases recorded, 33 letisimulated longest on the first trial, 11 on the second, 15 on the third, 2 on the fourth, 5 on the fifth, 7 on the sixth, 3 on the seventh, 5 on the eighth, 6 on the ninth, 5 on the tenth, 1 on the eleventh, 4 on the twelfth, 2 on the thirteenth, and 1 on the sixteenth. We have here, in a pronounced manner, the irregularity noticed by the Severins and Gee and Lathrop in the forms studied by them.

Effects of Temperature upon the Duration of Letisimulations.—To test this matter the 100 individuals mentioned were grouped according to the temperatures at which the experiments had been performed, and the results recorded in six tables. From the averages of those tables the following table was compiled.

TABLE SHOWING THE AVERAGES OF THE EFFECT OF TEMPERATURE ON THE LETISIMULATIONS.

Temperature in F. Degrees.	Number of Individuals Used.	Number of Letisimulations in Twenty Trials.	Maximum Time in Min. of a Single Letisimulation.	Number of the Trial on which Max. Let. was Made.	Total Time Consumed in Letisimulations.
60–65	7	15	3.35	4	14.19
65–70	12	16	6.86	2	26.11
70–75	17	17	7.25	5	35.11
75–80	23	13	3.97	5	13.06
80–90	17	12	5.91	6	13.30
85–90	21	9	3.83	6	12.15

If we were to rely upon these averages, we would conclude that up to 75° F. both the length of the maximum feint and the total duration of twenty feints vary directly with the temperature; and that beyond that point there is no definite relation between temperature and the feints. This conclusion, however, is not supported by a critical study of the individual records from which the averages were compiled. To test the matter further, four individuals were selected and each put through five series of twenty letisimulations, each series being conducted at a different temperature. The results were recorded in four tables. There was no obvious relation between temperature and the duration of the feints.

Effects of the Strength of Stimulus upon Letisimulation.—To get the stimulus as nearly uniform as possible, the ant-lion was gently shoved from a glass ledge and caused to fall three inches. To secure a strong stimulus the ant-lion was permitted to fall upon a glass plate; to secure a weak one it was allowed to drop on a layer of cotton batting. The results of experimenting with 100 ant-lions was tabulated. In 36 cases the first letisimulation following a strong stimulus was the longer and in 58 cases the first feint following a weak stimulus was the longer. In six cases the duration of the feint was the same for each stimulus. The average of 100 individuals gave the duration of the first letisimulation following a weak stimulus as of longer duration than the first following a strong stimulus. These data do not seem to warrant a conclusion.

Effects of Hunger upon Letisimulation.—Certain selected individuals were well fed and others were forced to fast for a long time before they were used for experiments identical with those mentioned above. The results were carefully tabulated. No relation could be detected between hunger and the length of the letisimulation.

Apparently the reason for the longest letisimulation being located sometimes at one place and sometimes at another in the series is due to some internal (physiological) factor not revealed by these experiments.

Weir[1] considers the letisimulation of animals "one of the greatest evidences of intellectual action, on their part." Hamilton (29), Webster (39) and a few others feel that the creatures consciously fear death and take this means to avoid it. Dr. Lindsley, in "Mind in Animals," thinks "this must require great command in those that practice it." However, the majority of modern students of the subject look upon it as merely a remarkable instinct.

No one who is acquainted with how slowly the ant-lion recovers from injuries could, for a moment, consider anything intellectual, which induces it to passively submit to portions of its legs and of its mandibles being amputated. The tonic contraction of the muscles and the diminished reflex irritability suggest hypnotic phenomena and lead one to agree with Holmes (31) that "the instinct of feigning death is doubtless connected with much of what has been called hypnotism in the lower animals." It is well known that most animals pause momentarily when confronted with an unexpected or violent stimulus. To me the letisimulation of the ant-lion appears to be such a pause prolonged and exaggerated. The more I ponder over the results of my experiments upon the death-feigning of the ant-lion, the more I feel inclined to exclaim with James: "It really is no feigning of death at all and requires no self-command. It is simply terror paralysis which has been so useful as to become hereditary."

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[1] Weir, "Dawn of Reason," 1889, p. 202.

Conclusions.

1. The pits of the ant-lion are not confined to sand; they may be found in any kind of dry friable soil, that is protected from the rain and from insect-eating creatures. They are usually in clusters; but, occasionally, a solitary pit is found. On yet rarer occasions, pits may be found that are not under a shelter.

2. The ant-lion of the Middle West has two methods of excavating its pits. Usually it furrows backward, excavating a series of concentric, adjacent circles, each deeper than the last, and shovelling out the soil with its head. The front of the body is so curved as to make it easy for the dirt to fall forward on the head. In the second method, the larva simply burrows downward into the ground and tosses out the soil with its head until the sides of the pit become approximately stable. Pits formed by the second method are usually subsequently enlarged.

3. The ant-lion removes a medium sized obstacle from its pit by inserting the tip of its abdomen under it and, with the burden poised on its abdomen, backing slowly up the slope.

4. After its trap has been completed, the ant-lion rests quietly, in practically a horizontal position, with its body beneath the soil and its open mandibles in the bottom of the pit.

5. Any invertebrate, be it insect, arachnid, or crustacean, that happens to fall into the trap is acceptable food. Some escape, but the larva attempts to capture all. When captured, the

victim is dragged partly or wholly beneath the soil, and the juices imbibed through the hollow mandibles. Later the dried carcass is tossed away.

6. The ant-lion may be considered positively geotactic, positively thigmotactic and negatively phototactic; with the reservation that all of its movements cannot be explained as tropisms in the Loebian sense.

7. It is impossible for the ant-lion to move forward; but, in its backward movements, it can move in straight lines or curves, and can scale vertical surfaces that are not too smooth. The hind legs assist in producing this backward movement, and the other legs brace the body.

8. Sometimes it avoids water and at others it backs into it.

9. If the spherical cocoon of this insect is near the surface of the ground, the chrysalis comes only part of the way out and the imago emerges from its back. If the cocoon is at a slightly greater depth, the chrysalis comes entirely out of the cocoon and part of the way out of the ground. If the cocoon is at a greater depth, the chrysalis emerges entirely from the cocoon and perishes on the way to the surface of the ground.

10. Rough handling or dropping from a slight elevation will usually cause an ant-lion to letisimulate. The length of a feint and the position of the longest feint in a series of successive feints varies in different individuals and in the same individual at different times.

11. There is no obvious relation between the temperature, the strength of the stimulus, or fasting and the duration of a letisimulation.

12. If the relative durations of the successive feints of a long series of letisimulations are plotted, the curve will have two or more crests.

13. In the ant-lion all letisimulation poses are not death attitudes. The ant-lion has no characteristic death-feigning posture. It is to be grouped with those insects in which the letisumation pose varies with the attitude of the individual at the time when the stimulating shock is received.

14. Although pinching a leg and, sometimes, even blowing on the body, will usually cause a letisimulating ant-lion to come out

of its feint, in the majority of the cases, it will submit to the clipping off of the tips of its legs and of its mandibles without responding in any visible manner.

15. In the ant-lion letisimulation seems to be but an exaggerated prolongation of the pause made by most animals when they are startled. The total behavior of a death-feigning ant-lion supports Holmes's contention that "the instinct of feigning death is connected with much of what is called hypnotism in the lower animals"; and endorses James, when he says: "It is really no feigning of death at all and requires no self-command. It is simply terror paralysis which has become so useful as to become hereditary."

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August 10, 1915.

THE EFFECT OF CERTAIN ORGANIC AND INORGANIC SUBSTANCES UPON LIGHT PRODUCTION BY LUMINOUS BACTERIA.

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While engaged in a study of the chemistry of light production by luminous bacteria I had occasion to investigate the effect of diluting the sea water with distilled water and with isotonic sugar solution and the influence of the various salts of sea water, of acids and alkalies, and of certain anæsthetics upon the emission of light. The results are of interest for comparison with the known effects of these substances on other organisms and with other vital manifestations of life.

In all experiments, except where otherwise noted, one drop of the dense emulsion of luminous bacteria (a form isolated from squid at Woods Hole, Mass.) was added to 30 c.c. of solution in an uncorked Erlenmeyer flask and the whole thoroughly mixed. For comparative observations it is essential that the eye be thoroughly adapted to the dark and that each flask be oxygenated by shaking, before judging as to the emission or absence of light. Observations were made after 10 minutes, one hour and 24 hours.

Table I.

Effect of Dilution of Sea Water with Water and with *m* Cane Sugar Solution.

Dilution with Water.					Dilution with *m* Cane Sugar.				
Parts Sea Water.	Parts Water.	Light after			Parts Sea Water.	Parts *m* Sugar.	Light after		
		10 Min.	1 Hr.	24 Hrs.			10 Min.	1 Hr.	24 Hrs.
2	1	+	+	+	2	1	+	+	+
1	1	faint	faint	—	1	1	+	+	+
1	2	faint	very faint	—	1	2	+	+	+
1	4	faint	—	—	1	4	+	+	faint
1	6	very faint	—	—	1	6	+	+	faint
1	10	—	—	—	1	10	+	+	faint
1	14	—	—	—	1	14	+	+	—
1	20	—	—	—	1	20	+	+	—
Sea water		+	+	+	*m* cane sugar		+	+	—
Water		—	—	—					

It will be noted from the above Table I. that the bacteria cease to give off light and experiment shows that they are killed by too great dilution with water. That this effect is not entirely due to the absence of salt but is chiefly due to a cytolysis through lowered osmotic pressure is shown by diluting the sea water with an inert isotonic solution, cane sugar. Some salt is necessary for the continued production of light as the bacteria no longer glow after twenty four hours' emersion in *m*-sugar, a fact of no great surprise as unicellular freshwater luminous animals are unknown.

Table II.

Effect of Acid and Alkali.

Conc. of Acid and Alkali Added to Mg-free Sea Water, $m/2$ (100 NaCl + 2.2 KCl + 2CaCl$_2$) in Syracuse Watch Glasses.	Light after		
	10 Min.	1 Hr.	24 Hrs.
n/2000 HCl	–	–	–
n/4000 HCl	faint	–	–
n/8000 HCl	+	faint	–
n/16000 HCl	+	+	–
n/32000 HCl	+	+	faint
n/500 valerianic acid	–	–	–
n/1000 " "	faint	–	–
n/2000 " "	faint	–	–
n/4000 " "	+	faint	–
n/8000 " "	+	+	–
n/16000 " "	+	+	faint
n/500 NaOH	–	–	–
n/1000 NaOH	–	–	faint[1]
n/2000 NaOH	+	+	+
n/250 methyl amine	–	–	–
n/500 " "	faint	–	faint[1]
n/1000 " "	faint	+[1]	faint
n/2000 " "	+	+	+
Mg-free sea water	+	+	faint
Sea water	+	+	+

[1] Probably due to neutralization of alkali through absorption of CO$_2$.

As was to be expected acids and alkalies prevent light emission in very weak concentration, the acids in much weaker concentration than the alkalies. In fact the bacteria are very sensitive to acid and will not even phosphoresce with any brilliancy in a neutral medium.

The organic acid (valerianic) and alkali (methyl amine) have less effect than the inorganic, a result at variance with my results for other organisms which are usually affected more readily by the weak than by the strong acids and alkalies.[1]

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[1] Harvey, E. N., "Studies on Acids," in Carnegie Institution Publications No. 212, p. 143, 1915; on alkalies, *id.*, No. 183, p. 131, 1914.

TABLE III.

EFFECT OF VARIOUS COMBINATIONS OF THE SALTS OF SEA WATER.

Salt Combinations.	Light after		
	10 Min.	1 Hr.	24 Hrs.
Sea water	+	+	+
Artificial Sea Water, $m/2$ (100 $NaCl + 2.2\ KCl + 2\ CaCl_2 + 10\ MgCl_2$) $+ n/4000\ NaOH$	+	+	+
Neutral artificial sea water	+	+	faint
$m/2\ NaCl$	+	+	faint
$m/2\ KCl$	+	faint	–
$m/3\ CaCl_2$	–	–	–
$m/3\ MgCl_2$	–	–	–
$m/2\ (100\ NaCl + 2.2\ KCl)$	+	+	faint
$m/2\ (100\ NaCl + 2\ CaCl_2)$	+	+	faint
$m/2\ (100\ NaCl + 10\ MgCl_2)$	+	+	faint
$m/2\ (100\ NaCl + 2.2\ KCl + 2\ CaCl_2)$	+	+	faint
$m/2\ (100\ NaCl + 2.2\ KCl + 10\ MgCl_2)$	+	+	faint
$m/2\ (100\ NaCl + 2\ CaCl_2 + 10\ MgCl_2)$	+	+	faint

The most interesting point brought out in the above table is the independence of these bacteria of a balanced medium. The bacteria live and phosphoresce in pure NaCl without the addition of any bivalent ions. This is true even when the solution is changed three times to remove the last traces of Ca in the bacteria. KCl is also relatively non-toxic, although more so that NaCl. $CaCl_2$ and $MgCl_2$ are very toxic when alone. All combinations of NaCl with the other ions of sea water sustain the bacteria well except that they are neutral media and hence the phosphorescence is dimmed after 24 hours. That pure NaCl should have so little effect on light production is astonishing when we consider its poisonous effect on other marine organisms and tissues, particularly on ciliated cells.

The effect of the alcohols (Table IV.) on light production is very similar to their effect on other life processes: they exert an inhibiting or anæsthetic action which is perfectly reversible. If alcohol solutions containing bacteria which have stopped emitting light are diluted with sea water, light production again begins. As with other tissues the higher the alcohol in the series the greater anæsthetic power it has.

The effect of a number of other substances was studied in a very rough way—namely, by adding a small quantity of the substance to a sea water emulsion of the bacteria in test tubes and then shaking the tubes. With toluol, benzol, ether, chloroform, carbon disulphide, carbon tetrachloride and ethyl butyrate

Table IV.

Effect of Alcohols.

Conc. of Alcohol Added to Sea Water.	Light after		
	10 Min.	1 Hr.	24 Hrs.
Methyl alcohol, 2*m*	–	–	–
HCH_2OH, 1.5 *m*	+	very faint	–
HCH_2OH, *m*	+	faint	–
HCH_2OH, *m*/2	+	+	+
HCH_2OH, *m*/3	+	+	+
Ethyl alcohol, *m*	–	–	–
CH_3CH_2OH, *m*/1.5	very faint	–	–
CH_3CH_2OH, *m*/2	+	faint	faint
CH_3CH_2OH, *m*/3	+	+	+
Propyl alcohol, *m*/3	–	–	–
$CH_3CH_2CH_2OH$, *m*/4	very faint	–	–
$CH_3CH_2CH_2OH$, *m*/6	faint	very faint	–
$CH_3CH_2CH_2OH$, *m*/8	+	faint	–
$CH_3CH_2CH_2OH$, *m*/15	+	+	+
Isosbutyl alcohol, *m*/10	–	–	–
$(CH_3)_2CHCH_2OH$, *n*/12	very faint	–	–
$(CH_3),CHCH_2OH$, *m*/16	+	very faint	–
$(CH_3)_2CHCH_2OH$, *m*/20	+	+	–
$(CH_3)_2CHCH_2OH$, *m*/24	+	+	+
Amyl alcohol, *m*/20	–	–	–
$C_2H_5CH_3CHCH_2OH$, *m*/40	–	very faint[1]	+[1]
$C_2H_5CH_3CHCH_2OH$, *m*/80	very faint	very faint	+[1]
$C_2H_5CH_3CHCH_2OH$, *m*/160	faint	+[1]	+
$C_2H_5CH_3CHCH_2OH$, *m*/320	+	+	+
Capryl alcohol, *m*/400	–	–	–
$CH_3(CH_2)_6CH_2OH$, *m*/800	very faint	very faint	–
$CH_3(CH_2)_6CH_2OH$, *m*/1600	faint	faint	–
$CH_3(CH_2)_6CH_2OH$, *m*/3200	faint	faint	+[1]
$CH_3(CH_2)_6CH_2OH$, *m*/6400	+	+	+
Sea water	+	+	+

[1] Probably due to evaporation of alcohol.

the light was found to disappear almost immediately; with tannin, chloral hydrate, vanillin and sodium glycocholate the light had disappeared in the course of one hour while saponine, amygdalin, and sodium taurocholate had no effect. It is surprising that saponin has no effect on luminous bacteria when we consider its great cytolytic power on other forms in very small concentration.

Summary.

The effects on luminous bacteria of dilution of sea water with water and *m* sugar solution; of HCl and valerianic acid; of NaOH and methyl amine; of the salts of sea water in different combinations; and of methyl, ethyl, propyl, butyl, amyl and capryl alcohol were studied. The points of interest in the results are indicated after each table.

FURTHER NOTES ON THE CHROMOSOMES OF THE CERCOPIDÆ.

ALICE M. BORING AND RAYMOND H. FOGLER.

The chromosomes in the spermatogenesis of five species of this family of Hemiptera have already been studied by Stevens[1] and Boring.[2] Three more species have now been studied in comparison with those previously studied. They are *Philaenus lineatus*, *Aphrophora parallela* and *Clastoptera proteus*. Each of these three species belongs to a genus in which one or more species has already been studied, so this gives a chance to compare the spermatogenesis in closely related species. This has been done very carefully by McClung[3] for some families of Orthoptera. The entire family Acrididæ has the same spermatogonial chromosome number, 23, and the Locustidæ has 33, but within each family there are generic and specific cytological differences. The family Cercopidæ of the Hemiptera does not show as closely graded a series of cytological differences as the orthopteran families studied by McClung. The facts as found are here recorded.

The material was collected at Woods Hole[4] and in Orono; *Philænus lineatus* from grasses, *Aphrophora parallela* from Scotch pines, and *Clastoptera proteus* from alders. Dr. Herbert Osborn has very kindly identified the species of the material. Flemming's and Gilson's solutions were used for fixation, and iron hæmatoxylin for staining.

Philænus lineatus has 29 chromosomes as spermatogonial number (Fig. 1), two of which are larger than the others. The odd chromosome is round or oval in the early (Fig. 2) as well as the late (Fig. 3) spireme stages. The reduced number of chromosomes is 15 in the first spermatocytes, one of which is larger than the others (Fig. 4). The odd chromosome is univalent (Fig. 5) and does not divide in the first spermatocyte division (Fig. 6). The second spermatocytes have partly 15 (Fig. 7)

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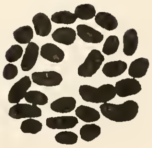
[1] N. M. Stevens, '06, "Studies in Spermatogenesis," Pt. II., Carnegie Institute, Washington.

[2] A. M. Boring, '07, "A Study of the Spermatogenesis in the Membracidæ," etc., *Jour. Exp. Zool.*, 4, p. 469. '13, "The Chromosomes of the Cercopidæ," BIOL. BULL., 24, p. 133.

[3] C. E. McClung, '08, "Cytology and Taxonomy," Kans. Univ. Bull. 4.

[4] We wish to thank the Director of the Marine Biological Laboratory for the privileges of the laboratory during the summers of 1913 and 1915, at which time this material was collected.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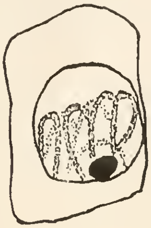
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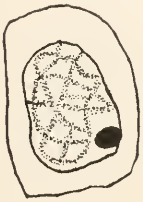
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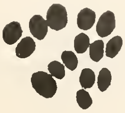
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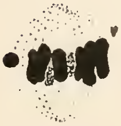
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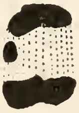
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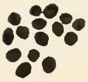
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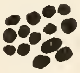
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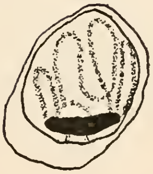
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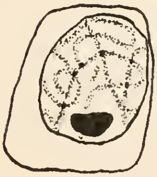
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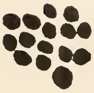
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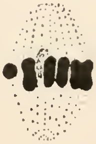
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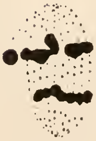
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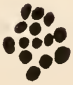
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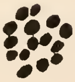
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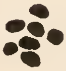
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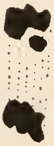
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and partly 14 (Fig. 8) chromosomes. The chromosome number is specific, as the reduced number is 15, while only 12 are found in *Philænus spumarius.* But the roundness of the odd chromosome throughout the spireme stages is a feature common to both species of this genus, and distinguishing it from the species of the genus *Aphrophora.*

Aphrophora parallela has 15 chromosomes as reduced number, with one largest chromosome (Fig. 11). The odd chromosome is elongated in the early spireme stages (Fig. 9) and becomes more nearly round in the later stages (Fig. 10). The odd chromosome is, as usual, univalent (Fig. 12) and does not divide in the first spermatocyte division (Fig. 13). The chromosome number in the second spermatocytes is 14 and 15 (Figs. 14 and 15). Again in this species, the chromosome number is different from that in the other species of the same genus, that is, 15, in comparison with 14 in *Aphrophora quadrinotata* and 12 in *Aphrophora spumaria.* The long odd chromosome in the early spireme stages is a common feature of both *A. spumarius* and *A. parallela,* and distinguishes them from the genera *Philænus* and *Clastoptera.* The early spireme stages of *A. quadrinotata* were not studied. The species formerly classified as *A. quadrangularis* has since been put into the genus *Lepyronia.* This species does not possess the long odd chromosome characteristic of the genus *Aphrophora.*

Clastoptera proteus has 7 as reduced chromosome number (Fig. 16), one less than the reduced number in *Clastoptera obtusa.* Unfortunately only a few stages were found in this material, so that the only other significant point that was observed was that the first spermatocyte division is the one in which the odd chromosome does not divide (Fig. 17) as in all the species of this family.

TABLE I.

Genus.	Species.	Reduced Chromosome Number.
Philaenus	*spumarius*	12
"	*lineatus*	15
Aphrophora	*spumaria*	12
"	*quadrinotata*	14
"	*parallela*	15
Lepyronia	*quadrangularis*	11
Clastoptera	*proteus*	7
"	*obtusa*	8

The eight species of *Cercopidæ* in which the spermatogenesis has so far been studied belong to four genera. The chromosome number (reduced) varies from 7 to 15. The chromosome number seems to have no significance for family or genus. The specific numbers are shown in Table I.

The odd chromosome in the spireme stages differs in its shape in the genus *Aphrophora* from that in the other genera as far as studied. It is a much elongated structure early in its appearance in *Aphrophora*, while it first appears as an oval or round body in the others.

All eight species of the Cercopidæ studied show a typical odd chromosome, which divides only in the second spermatocyte division. In all of the species except *Aphrophora quadrinotata* and *Clastoptera proteus*, in which the material was limited and the equatorial plates consequently not studied in favorable positions, there is one chromosome among the reduced number which is distinctly larger than the others. In no case is the odd chromosome the largest one.

WOODS HOLE,
July 30, 1915.

THE REACTIONS OF AN ORB-WEAVING SPIDER, EPEIRA SCLOPETARIA CLERCK, TO RHYTHMIC VIBRATIONS OF ITS WEB.[1]

WILLIAM MORTON BARROWS.

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I. INTRODUCTION.

The work reported in this paper was suggested by a chance observation[2] made in the summer of 1911. A fly was held close to one of the spiders without eliciting any response; when the fly's vibrating wing was allowed to touch a strand of the web, however, the response was instantaneous and positive. The spider ran to the fly and seized it. A vibrating rubber band held against a strand of the web caused a very similar response. During the summer of 1913 these spiders were studied more carefully in an attempt to determine: first whether the stimulus was vibratory in nature or must be considered to be due to some other force and second whether the response could be identified as a "tropism" or taxis.

II. MATERIALS AND METHODS.

At the Lake Laboratory maintained by the Ohio State University at Cedar Point, Ohio, the large orb weaving spider, *Epeira sclopetaria*, is very abundant, building its webs on the front porch and in the angles of the building and roof. The habit of the female of this species of remaining at the center of her web for long periods of time makes it a very convenient form to study in its normal surroundings.

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[1] Contribution No. 42 from the Department of Zoology and Entomology, Ohio State University.

[2] The note of Boys (80) was not known to the writer until the larger part of these experiments had been carried out.

This species builds its web in dead branches or in the angles of buildings where there is an abundance of small or medium sized insects. The web usually consists of 17, 18 or 19 relatively inelastic strands which radiate from a center like the spokes of a wheel. These radiating strands are attached at their outer ends to twigs or boards or to guys or stays which anchor several radii to the support. Surrounding the center of the web is an irregular network known as the hub and notched zone which serves as a resting place for the inhabitant of the web. For a short space outside the hub the radii are bare (the free zone) but beyond this is found the viscid spiral consisting of finer strands which are extremely elastic and are beaded with microscopic sticky drops which serve to hold and entangle the insect prey. It is probably the extreme elasticity of these spiral strands which allows them to detain a strong insect without being snapped, thus giving the spider time to reach the detained insect and complete its ensnarement by the addition of fresh silk from the spinneretts. The normal resting position of the female spider is with the head directed downward and the legs spread outward on the notched zone as is shown in Fig. 1. The method used to obtain this photograph and the others following is that used by Comstock ('12, p. 181). A female spider was placed on a dead branch held in the neck of a bottle which was set in a tray of water. During the first or second night the spider usually built a perfect web. The branch was then moved to the photographing table with as little disturbance as possible and placed in front of a soap box painted a dull black on the inside. Arranged in this way before the camera it was possible to take pictures showing the spider, web, and vibrator straw, and the various positions taken by the spider in the act of responding to the vibrator.

The size of the web varies from two inches in diameter, or even smaller when built by very young spiders, to eighteen inches or more when built by mature females. The male builds a web very much like that made by the female but as he has a roving disposition one is never sure that the same individual can be located twenty-four hours later while the females often live for weeks in the same place, repairing the web every evening but not altering it materially.

In crawling across the web the spider always follows a radiating strand or at the edge of the web, one of the guy strands, and places its feet on the radii or on the junctions of the radii and spiral threads where the latter hold no sticky materials. The front feet are usually placed on the same radiating strand but the second and third pairs may be spread out on the two adjoining strands. It is possible for the spider to crawl rather swiftly along a single strand for a considerable distance, all eight feet using the same thread. In crossing the web the spider usually leaves behind a dragline which may remain across the web, adhering to it after the spider has returned to the center Some individuals on the other hand when they reach the edge of the web swing free, held only by the drag line up which they climb in returning to the center. Occasionally one finds both methods employed by the same individual. Most spiders are not skilful enough to cross the web several times without tearing out or snagging several of the segments of the spiral thread. When the web is violently disturbed the spider usually retreats to a niche or corner (the retreat) and remains there motionless unless again disturbed. Some individuals remain in the retreat instead of at the center of the web. When this is done one forefoot is placed on the trapeline leading to the hub and any activity of the web such as that produced by an entangled insect sends the spider like a flash down to the web. In this connection another fact may be noted; a spider outside the center of the orb always returns to the center, takes the normal position and then orients before it finds an entangled insect. This might be explained as due to the difficulty of crossing the web by any other path than by the radii. However, the inability to orient accurately in any other position than the center gives a clue to a more probable explanation. Individuals of the species *Epiera sclopetaria* will eat nearly any insects which happen to become entangled in the web. The food of those studied inside the screened porch consisted almost entirely of rather large flies of the genera *Musca*, *Sarcophaga*, and *Lucilia*. It is in the snaring of these flies that this *Epeira* seems to be especially expert. When a fly strikes a web it often goes through, breaking out one or two spiral segments. If, however, it does not break through it hangs for a second, buzzing, then breaks one or two of the sticky strands and flies away. A fly seldom entangles itself to such an extent that it cannot get free inside of five seconds. A successful spider then must reach the fly in less than two or three seconds after it strikes the web. The actual capture of the fly is accomplished usually either by biting the fly and stunning it or by winding it with web. The entangled fly may be left where it struck or may be torn from the web, and carried attached to one of the spider's hind feet to the center of the web where it is thoroughly chewed and its liquid parts swallowed.

The apparatus used to produce rhythmic vibrations consisted of three tuning forks and an electric vibrator. One fork had a vibration rate of 100 double vibrations per second, another a rate of about 487 and the third was an adjustable fork with a large range of vibration rates but with very limited amplitude. The electric vibrator was a modified electric door bell in which the clapper was replaced by a long grass straw. The number of vibrations produced by this instrument could be varied to some extent by changing the tension of a spring and a regulator screw, while the amplitude of the vibration varied with the length of straw used. The vibration rate of the vibrator was obtained by comparing a tracing made by it on a sheet of blackened paper on a revolving drum with a simultaneous record made by the tuning fork giving 100 double vibrations per second. The electric vibrator was found to be more effective than a fork because it gave vibrations of equal intensity, *i. e.*, it did not run down. It had also another advantage in that it could be controlled by a switch held in the hand and could be operated at a distance from the operator. A stop watch was used to measure the time elapsing between the beginning of the stimulus and the arrival of the spider at the place where the straw touched the web.

III. Experiments.

1. *Experiments Using Rhythmic Vibrations.*

When the vibrator straw is placed against one of the spiral strands or against one of the radii and caused to vibrate the spider orients instantly and advances along the nearest radius to the straw, seizes the straw with its mandibles and may spread web on the straw with the hind pair of feet (Fig. 3). This reaction is carried out in essentially this manner no matter where the straw may strike the web.

The orientation is so rapidly executed and is followed so closely by the forward locomotion that it is difficult to separate the two parts of the response. If, however, the vibrator is set in motion for a fraction of a second only the orienting is accomplished but the forward locomotion toward the vibrator does not follow. A second vibration while the spider is oriented calls forth the forward response and an attack on the vibrator (Fig. 3). The photograph reproduced in Fig. 2 shows such an orientation. If the first vibratory stimulus is not too long or is not followed by a second stimulus the spider usually returns to the resting position at the end of a few seconds. Some individuals, however, follow the orienting response by an interesting series of activities. The fore feet are placed on neighboring radii, drawn toward the animal's body and released suddenly. This release sets the web vibrating parallel to the spider's longitudinal axis. The spider then turns one space to the right or left and repeats the process until she has oriented through a complete circle and set every pair of radii in motion. The use of this activity is seen if there happens to be a captured fly or a piece of dirt in the web. When the two radii which pass on either side of the object are set vibrating the object is also set in motion but its motion is not of the same rate as that of the rest of the web and it sets up an echo or return vibration. To this the spider responds. A dead fly may be rediscovered in this way or a piece of dirt may be located and removed.

Responses to different frequencies show considerable variations and it is not possible to predict that a certain individual will respond in a definite way to a given stimulus. This variation in response ranges from instantaneous orientation and forward locomotion to a slow orientation and slow approach toward the vibrating point or it may happen that no sign will be given that the stimulus has been perceived. Roughly speaking a large spider responds most quickly to a vibration of considerable amplitude with a vibration rate of 24 to 300 per second. It was impossible with the materials at hand to construct a vibrator giving a high rate and having also a considerable amplitude, so recourse to steel wires and small forks was necessary. The large spiders did not respond well to wires and forks with high vibration rate and small amplitude but they did respond instantly to the vibrating wings of *Chrysops* (127 per sec.), *Microbembex* (208 per sec.), *Musca* (284 per sec.), where the amplitude ranged from 4 mm. to 10 mm. Small spiders responded quickly to vibrations ranging from 100 per sec. to 487 per sec. and even higher although the amplitude was very small. This difference in responsiveness between the young and old spiders is probably correlated with differences in size and rate of wing vibration of the insects which are ensnared and used as food by young and old. In general small insects have high wing vibration rates while the larger insects have lower rates of wing vibration (Packard, '03, p. 150). The smaller spiders eat small insects and the large spiders eat larger insects. The following species of insects were caught and eaten by *E. sclopetaria: Chrysops vitatus* (127 vibr. per sec.); *Calliphora vomitaria* (130 vibr. per sec.); *Microbembex monodonta* (208 vibr. per sec.); *Musca domestica* (284 vibr. per sec.). Many small midges (*Chironomus* and others) were eaten by the young spiders and occasionally by the adults. The vibration rate of these small midges is probably very high, judged by the high pitched note which they give out, but it was impossible at the time to determine its rate.

2. *Experiments Using a Y-shaped Vibrator.*

In order to determine whether the spider reacted to a single vibrating strand or to the center of a vibrating area of the web, a Y-shaped vibrator made up of insulated magnet wire was adjusted to the vibrator and arranged in such a manner that its ends touched the web at two places, 2 or 3 cm. apart. When the vibrator so adjusted was operated the spider responded readily, going to a point on the edge of the web midway between the two vibrating points and then after some slight hesitation going toward one or the other of the vibrator wires (see Fig. 4). If, however, these points of wire were more than 3 cm. apart the spider at the center of the web usually hesitated, turning first toward one, then toward the other, finally orienting to one and attacking this by itself.

3. *Response in the Dark.*

In order to test the ability to respond in the dark the vibrator was set up late in the afternoon, the straw touching one of the radial strands of a web which was built in the frame of a window. The window was shaded on the outside by a heavy thicket. At 9:30 P.M. the room was so dark that a person standing inside could discern the outline of the window with the utmost difficulty. A flash of light from a pocket electric lamp showed that the female occupying the web was at the center of her web. The vibrator switch was closed and at the end of about four seconds the electric flash light showed the spider biting the vibrator straw in the same manner as that shown in Fig. 3. This experiment indicates that unless these spiders use rays of light which our eyes do not perceive, sight plays no essential part in the orientation to and the ensnaring of the prey.

4. *The Distribution of Vibrations through the Web.*

The distribution of vibrations as they travel across the web is of some theoretical interest. The following method for recording these vibrations was used with considerable success. A spider in its web was placed before the camera and made to respond to the vibrator repeatedly until it would respond no more. A photograph (Fig. 5) of 15 seconds' exposure was then made while the vibrator was in motion. The web was somewhat torn by the spider before it ceased to respond, but the photograph reveals by the thickening of the lines the distribution and amplitude of the vibrations in all parts of the web. The amplitude of the vibrations decreases rapidly from the periphery toward the center. The radial strand connected with the vibrator shows the greatest lateral displacement while the strands on either side of this show less and less disturbance as the distance away from the vibrator increases. A slight thickening of the spiral strands in a direction at right angles to the direction of the primary vibration can be noted on the segments directly across the center from the vibrator. The center of the web seems to be the part least affected. If there is any motion here it is probably at right angles to the original vibration, that is, it is probably parallel to the spiders' long axis after orientation.

5. *Mutilation Experiments.*

The foregoing experiments coupled with careful observations on the spinning behavior of the orb-weaver lead to the conviction that the organs used in detecting the movements of the web are proably tactile, at least there are no other organs described which would seem to serve the purpose as well. There can be little doubt that sense hairs are very abundant on the legs, particularly on the tarsi of these spiders. These hairs have been described by Dahl (83), Wagner (88), McCook (90), and recently by McIndoo (11). The functions of these hairs have been interpreted in various ways, but little or no experimental work has been accomplished other than attempts to show that some spiders hear. Responses to sounds seem to have been observed only in those forms which build webs. It seems likely that responses in the web building forms are due to the vibrations of the air being picked up by the strands of the web (McIndoo, '11, p. 412). It was thought desirable to determine if possible the location of the sense-organs used in detecting vibrations. By careful manipulation with a pair of fine dissecting scissors it was possible to snip off one or more of a spider's legs without causing the spider to leave the web. It is necessary to use great care not to shake the web because an irregular shaking gives rise to the negative response, the spider running away to the retreat. The contrast between this insensibility to the amputation of legs and extreme sensitiveness to irregular vibrations of the web emphasizes the fact that these spiders receive most if not all of their mechanical stimuli through the web. These operations caused the spider to lose considerable blood but two or three hours usually sufficed to heal the wound. The stumps of the legs were always held up so that they did not touch the web.

Experiment 1.—After testing a spider to be assured that its responses were normal the two forelegs were cut off as near the middle of the metatarsus as possible. This spider immediately put the stumps of the forelegs into its mouth. The next morning this spider was in its web. During the night the web had been repaired and a new spiral thread put on.

In recording the test made on this spider and those following, IX o'clock, XII o'clock, etc., refers to the position at the edge of the web which corresponds to the same hour on the clock face. Thus VI o'clock is used to designate the edge of the web which the spider normally faces when at rest, *i. e.*, directly downward.

Experiment 1.—Spider with both forefeet cut off. Fork giving 100 vibrations per second touching web in

III o'clock position spider reached fork 8 inches from center in 3 seconds.

IX o'clock position reached fork (8 in.) in 2½ sec.

VII o'clock position reached fork (8 in.) in 2½ sec.

Experiment 2.—Spider with third legs cut beween femur and patella.

IX o'clock position reacted 8 in. in 2¼ sec.

III o'clock position reacted 8 in. in 2¼ sec.

XII o'clock position reacted 8 in. in 7¼ sec.

This individual showed some difficulty in climbing, but oriented accurately.

Experiment 3.—Spider with second legs cut off at patella.

III o'clock position reacted 7 in. in 1½ sec.

XII o'clock position reacted 7 in. in 1½ sec.

X o'clock position reacted 7 in. in 1½ sec.

Experiment 4.—Spider with fourth legs cut off at patella. Reactions entirely normal as given above.

Another set of experiments which need not be detailed were carried out. In these the right first leg and left fourth leg were cut off and other similar combinations were made. In all cases orientations and the locomotion following were entirely normal except for the slight difficulties in locomotion which might be expected. These experiments indicate that the sense organs used in reacting to the vibratory stimuli are not restricted to any one pair of legs below the metatarsus. There are two possible distributions of sense hairs which would seem to make possible the reactions detailed above; the sense organs may be confined to the feet, where they come in contact with the web or they may be located on the legs or body in such a manner that they pick up the vibration of the whole leg or whole body. Hinged sensitive hairs uniformly scattered over the body might answer this purpose. It seems most likely, everything considered, that the particular sense organs used are on the tarsi of each leg and come in contact with the web. It is difficult to conceive that an animal whose feet are not extremely sensitive could travel on or manipulate the delicate strands of these orb-webs.

IV. Discussion and Summary.

It is maintained in this paper as in a previous one (Barrows, '07) that an animal exhibits a "tropism" or better a taxis, "when under the influence of [chemical] stimuli acting unilaterally they move toward or away from the source of the stimulus" (Verworn, '99, p. 249). It has been shown above that *Epeira sclopetaria* orients in its web and moves toward the source of a vibratory mechanical stimulus when this is of an appropriate rate and amplitude. Thus this method of response to a vibratory stimulus identifies the reaction as a positive taxis. The term tonotaxis would naturally be used in this connection, but since tonotaxis has been used in another way it seems advisable that the terms positive vibrotaxis should be applied if a short descriptive term is desired.

The foregoing may be summarized as follows:

1. *Epeira sclopetaria*, an orb-weaving spider, starting from the center of its web is able to orient, charge and seize flies which strike and are detained in the web. This process is carried out with extreme rapidity.

2. With the aid of a mechanical vibrator it is possible to show that the stimulus is vibratory, the spider orienting to and attacking the vibrator even in the dark.

3. The response can be analyzed into, (*a* the orientation, (*b*) the forward response, and (*c*) the attack on the vibrating object. The response is in essence a positive vibrotaxis.

4. The vibrations are transmitted through the web in all directions from the vibrating point but the intensity (amplitude) decreases toward the center of the web and on either side. The lines of equal intensity of the vibration form roughly a series of circles the centers of which are at the vibrating point.

5. The sense organs used in detecting the stimulus are probably sense hairs on the tarsi.

6. This orb-weaving spider provides itself with a temporary extension of its tactile sense organs which makes its tactile sense in reality a distance receptor, much like an auditory or an olfactory organ.

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EXPLANATION OF PLATE I.

FIG. 1. Showing a female *Epeira sclopetaria* in the normal resting position in the web. The arrow indicates the place where the vibrator straw touches a radial strand of the web.

FIG. 2. The same individual, shown in Fig. 1, orienting to the vibrator which had been in motion for a fraction of a second just before the photograph was taken.

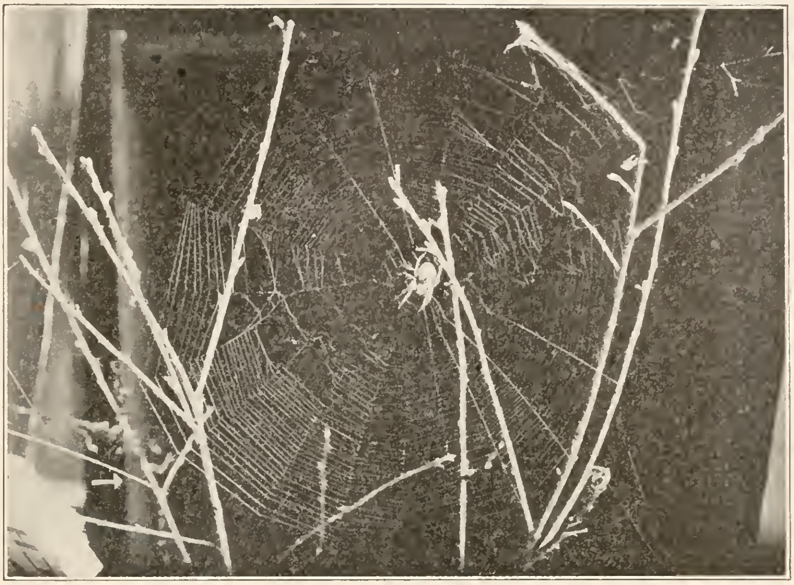

FIG. 1.

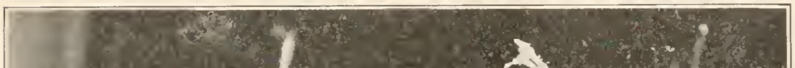

FIG. 2.

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EXPLANATION OF PLATE II.

FIG. 3. A spider attacking the vibrator straw while it is in motion.

FIG. 4. A spider in the act of responding to the Y-shaped vibrator. One prong of the vibrator appears in front, the other behind the spider.

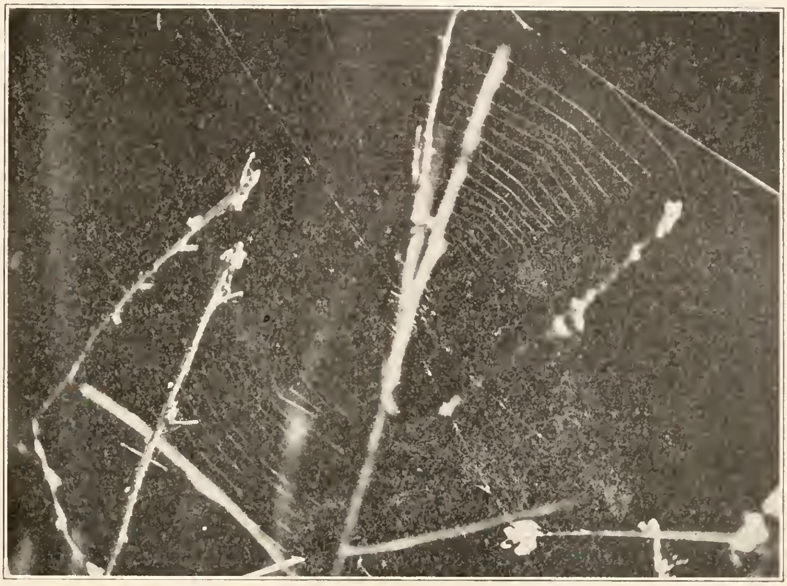

FIG. 3.

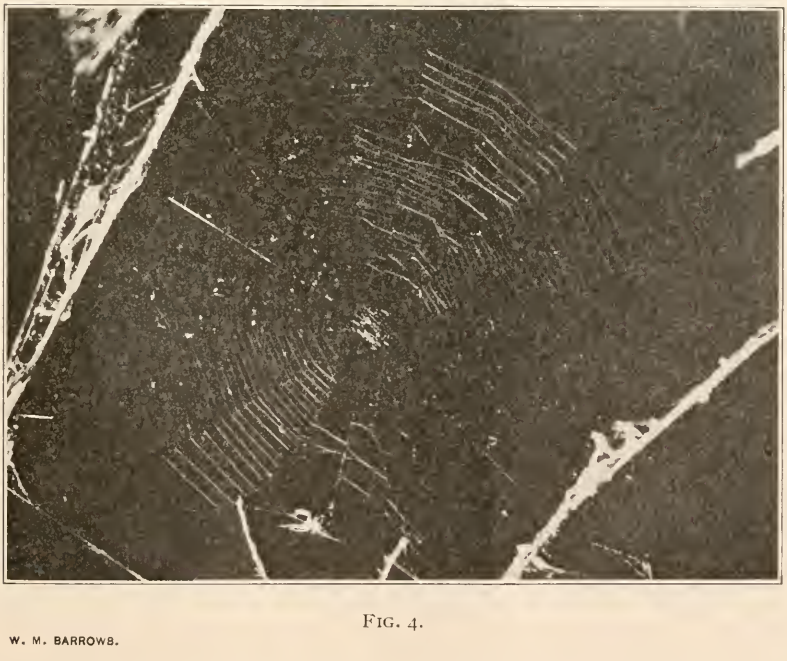

FIG. 4.

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EXPLANATION OF PLATE III.

FIG. 5. A photograph showing the spider in the normal resting position in the web, while the vibrator is in motion. The arrow indicates the place where the vibrator straw touches a radial strand. The doubling or blurring of the lines of the web shows the distribution of the vibrations.

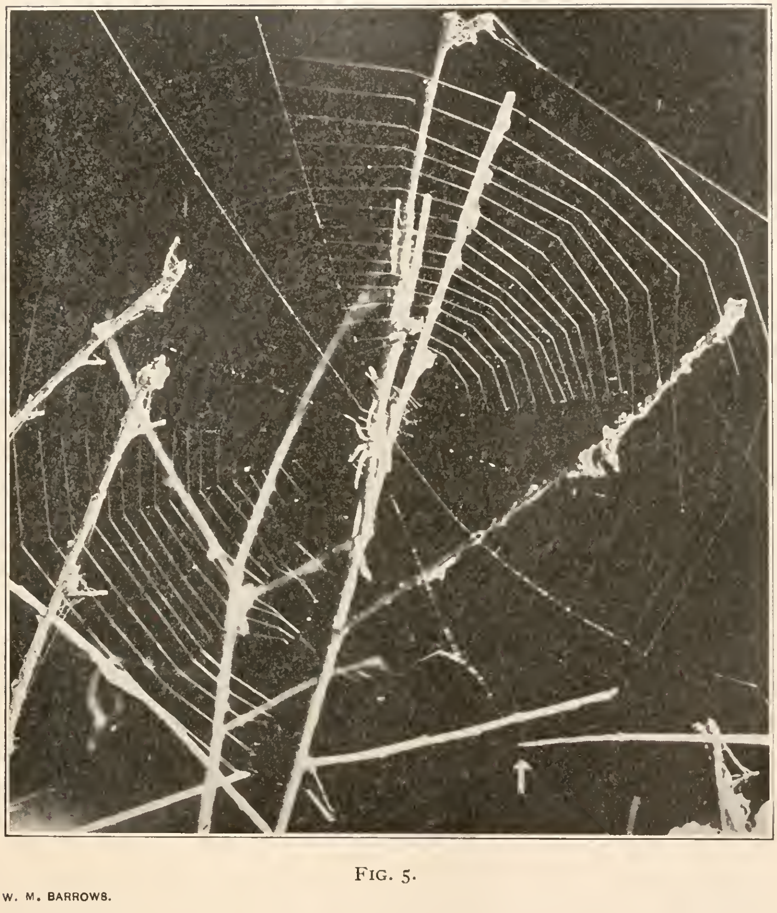

FIG. 5.

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BIOLOGICAL BULLETIN

OBSERVATIONS ON THE DEVELOPMENT OF COPIDOSOMA GELECHIÆ.

J. T. PATTERSON.

From the Marine Biological Laboratory, and the Zoological Laboratory of the University of Texas (Contribution No. 127).

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I. INTRODUCTION.

The discovery of polyembryonic development among certain of the hymenopterous parasites has opened up an extremely interesting field for investigation. Like most other important biological discoveries, this one was foreshadowed by the observations of several different naturalists. In a paper of this nature it is not necessary to give an extended account of the history of this discovery. We shall therefore be content with a brief statement on this point, limiting the account almost entirely to the species with which the paper deals.

The general features of polyembryony in insects have been given in the well-known papers of Marchal ('98, '04) and Silverstri ('06, '08), but there are many points concerning the details of this process which have not as yet been worked out. It was with the view of studying certain of these details that led the writer three years ago to seek an American species upon which such studies could be made. Dr. L. O. Howard[1] suggested that *Copidosoma gelechiæ*, which parasitizes the larvæ of the *Solidago* gall moth, *Gnorimoschema gallæsolidaginis*, would be a good form upon which to work, as it seemed to be an undoubted case of polyembryony.

The *Gnorimoschema* moth makes the ellipsoidal galls on the stems of several species of goldenrod. Baron Osten Sacken ('63) seems to have been the first to have published a description of the inflated carcass of the *Gnorimoschema* larva, caused by the chalcis parasite; but apparently he was not acquainted with the maker of the gall. In 1869 in connection with his account on the life history of this moth, Riley states that the caterpillar serves as a host for no less than six different species of hymenopterous parasites. One of these, which is shown in his Fig. 6, Plate 2, is described as a "little fly of a dark metallic green color, with reddish legs." This is clearly *Copidosoma*. Riley states that the larvæ of this species infests the caterpillar in great numbers, more than 150 having been obtained from a single host. He supposed that the "mother fly" gnawed a passage through the gall and desposited her batch of eggs in the inmate. He pointed out that the larval parasites cause the caterpillar to swell to three or four times its natural size, and after having absorbed all the juices of the victim, form very small brownish cocoons, which are so packed together that they give to the worm the puffed-up appearance which is typical of the mummified carcasses of lepidopterous larvæ that have been parasitized by a polyembryonic species. It was this inflated condition of the larval host that led Riley to call the parasite the "Inflating Chalcis

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[1] For this as well as for other suggestions received throughout the progress of the work, the writer is greatly indebted to Dr. Howard.

Fly." Howard ('85) later named this species *Copidosoma gelechiæ.*

Upon examining the various goldenrods about Woods Hole, Mass., for galls of *Gnorimoschema*, it was found that *Solidago sempervirens* furnished the best opportunity for obtaining material. However, the common gall maker of this solidago proved not to be *Gnorimoschema gallæsolidaginis* Riley, but a closely related species, *G. salinaris* Busck. The parasites infesting these two moths are varieties of the same species, *Copidosoma gelechiæ.*

The selection of this species has not proved altogether satisfactory, because the gall-making habit of the host complicates the life history and renders the collecting of material for early stages of the parasite somewhat more difficult than from a host which feeds openly. Furthermore, the moth, and likewise the parasite, has but one generation a year. In addition to these objections, there is the further one that the egg of *Copidosoma* gives rise to a relatively large number of individuals (about 191 on the average). In attempting to obtain material for the studies which the writer has in mind, it seems best to seek to find a host which is an open or semi-open feeder, which has two or more generations a year, and which harbors a parasitic egg giving rise to but few individuals. During the past summer at least two species have been found which in the main seem to fulfill these conditions. It therefore seems best to publish the main facts concerning the development of *Copidosoma* before giving it up for more favorable material.

There is one feature in the development of *Copidosoma* which makes further study desirable. We refer to the abortive embryos (presently to be described), which at first were thought to be comparable to the so-called asexual larvæ of *Litomastix truncatellus*. It will be recalled that Silvestri ('06) described in this species the development of both sexual and asexual larvæ from a single egg. In one instance he secured from a caterpillar of *Plusia gamma* 1,700 sexual and 220 asexual larvæ of *Litomastix.* He believes that the asexual larvæ play the rôle of raspers for the normal larvæ, tearing the tissues of the host so that the sexual larvæ may the more easily secure the necessary food. It may be stated here that the abortive larvæ of *Copidosoma* are in no way comparable to the asexual larvæ of *Litomastix* as described by Silvestri.

II. Note on the Life History of Gnorimoschema.

In order to collect polyembryonic material it is essential to know something about the life history of the host; especially is this true in cases like *Gnorimoschema* in which the larval host is a gall maker. Considerable attention has therefore been given to a study of the life history of *G. salinaris*.

The general habits of the *Solidago* gall moths were first made known by Riley's ('69) studies on *G. gallæsolidaginis*. According to Riley this species winters over in the imago stage and may be seen flying in the month of May. When the young plants (*Solidago nemoralis*) are about six inches high the female moth lays her egg either in the terminal bud or at the side of the stalk immediately below the bud. The young caterpillar upon hatching burrows into the stalk and starts the development of the gall. By the first of June the gall has just begun to form and contains a larva about one-third grown. The larva and its ellipsoidal gall reach their full size by the middle of July. The caterpillar which now measures over half an inch in length prepares for the change into the chrysalis state by first eating a round passageway through the wall well toward the upper end of the gall. The orifice is then closed by a secretion of liquid silk, which hardens to form a silken plug. After closing the orifice, the caterpillar lines the passage-way and the walls with a delicate silk, and then transforms into a shiny, mahogany-brown pupa, about a half inch long. The moths begin to emerge about the middle of August and continue to appear until the beginning of October.

Many phases of the life history of *G. salinaris* are similar to those of *G. gallaesolidaginis*, but there are some important differences. The earliest date at which galls of the marsh goldenrod have been secured was June 12, 1914, and at that time many of the galls were well started. Between June 12 and 15, 63 galls of various sizes were collected and examined. They varied in size from 8 to 12 mm. in length and from 4 to 17 mm. in transverse section. In shape the galls also vary greatly. Some are distinctly pear-shaped, while others are fusiform, with various gradations between these two general types. The galls occur at different heights on the stem, but the vast majority of them are located at or near the base of the stalk (Fig. 1). Their position is undoubtedly determined by the location of the point at which the larva penetrates the young shoot. If this point is located toward the base of the young stalk, the gall will naturally appear near the base of the fully grown plant; but if it is located in or near the terminal bud, the gall will appear some little distance up on the stem. Occasionally two galls are found on the same plant (Fig. 8). A few cases have been observed in which the gall was located at the tip of the terminal bud, producing a stunted plant without a central, flower-bearing stalk. With these few exceptions, the gall of *G. salinaris* does not seem materially to affect the growth and vigor of the plant. It is true that many galls are found on plants that are apparently stunted but such dwarfing is to be attributed to the adverse conditions under which the plant sometimes grows. In regions that are very much exposed to the wind, like the banks along the coast, many of the goldenrods are small and clearly dwarfed; but this condition applies as well to the plants that are free from galls as to those that are infected.

The habits of gall making are similar in the three common species of *Gnorimoschema*, although the following differences may be pointed out. *G. gallæasteriella* produces a triangular gall at the top of the dwarfed or stunted stems of *Solidago cæsia*, *S. axillaris*, *S. latifolis*, and *Aster divaricatus*.[1] The form of the gall differs somewhat with the plant. The gall of *G. gallaesolidaginis* may occur toward the top of the stem, but usually it is located just below the middle, especially is this true of the galls on *S. canadensis*. The galls of this moth do not dwarf the plant. The condition of the galls of *G. salinaris* on the marsh goldenrod has already been described. They occur nearer the base of the stem than do those of last species, and like the latter there is little or no tendency to dwarfing the plant.

The larvæ secured from the galls collected between June 12 and 15 varied from 3 to 8 mm. in length. Beginning with the middle of June, the young caterpillars grow rapidly, doubling their size within a fortnight. By the middle of July they have reached their full growth, and are beginning to show signs of undergoing pupation, which is evidenced by the construction of the passage-way. The passage-way and its orifice differ in two respects from those of *G. gallæsolidaginis* as described by Riley ('69). The silk lining does not extend much beyond the lower limits of the passage-way, and hence does not cover the inner surface of the wall. The second difference is seen in the character of the orifice and its silk plug. The caterpillar of *G. salinaris* does not cut the passage-way quite through the wall, but leaves the very thin epidermis of the stem, which is used as a background for the construction of the plug (Fig. 7).

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[1] Part of these data were kindly furnished the writer by Dr. T. M. Forbes.

TABLE I.

TABLE SHOWING DATES OF PUPATION AND EMERGENCE OF COPIDOSOMA AND GNORIMOSCHEMA.

Pupation (Beginning of)	Copidosoma	Aug. 6, 1912.
		Aug. 5, 1913.
		July 31, 1914.
		July 30, 1915.
	Gnorimoschema	Aug. 6, 1912.
		July 23, 1913.
		July 30, 1914.
		July 26, 1915.
Emergence	Copidosoma	Aug. 25 to Sept. 12, 1912.
		Sept. 3 to Sept. 13, 1913.
		Aug. 30 to Sept. 18, 1914.
		Aug. 24 to Sept. 21, 1915.
	Gnorimoschema	Aug. 25 to Sept. 10, 1912.
		Aug. 25 to Sept. 10, 1913.
		Aug. 22 to Sept. 11, 1914.
		Aug. 24 to Sept. 14, 1915.

Pupation occurs during the last week of July and the first week of August (Table I.). The imagines begin to emerge about August 25, and continue to appear until September 10. The moth has been seen flying in the open during this period.

Females kept in captivity often lay eggs. This they do within ten days after emerging, and irrespective of their association with males. As a rule the moths simply drop the eggs on the bottom of the cage, or they may lay them on the leaves and flowers of goldenrods placed in the cage. At first it was thought that *G. salinaris* must differ from *G. gallæsolidaginis* in respect to its egg-laying habits, for Riley states that the latter species although emerging in the fall, hibernates as an imago and lays its eggs in the following May. It has been discovered, however, that *G. gallæsolidaginis* from the galls of *S. canadensis* in western Ohio likewise drops several eggs soon after emerging from the pupa in September. This raises the question as to whether these fall eggs develop into larvæ, for if so it would be difficult to explain how the young caterpillars could withstand the winter and succeed in the spring in finding a young goldenrod bud or shoot in which to start the new gall.

In reply to an inquiry, Mr. A. Busck of Washington kindly informed the writer that the laying of eggs by *Gnorimoschema* was of no particular significance, as it is not uncommon for certain Lepidoptera to drop their eggs prematurely, especially if kept in captivity. In view of this fact an observation made in the fall of 1913 is of special interest. During the first few days of September of that year a single female, confined in a cage with several males, laid a dozen or more eggs on goldenrod leaves and flowers. On the thirteenth of the month three larvæ hatched from this batch of eggs! There can be no possible doubt as to the correctness of this observation, for the hatching of one of the little caterpillars was actually observed under a hand lens.

It is difficult to explain the development of these larvæ from fall eggs, except on the basis of parthenogenesis. It is true that the female which laid the eggs from which the larvæ developed had been confined with males; but although males and females have been kept together for several weeks during each of the last three seasons, yet mating has never been observed. The supposition that the fall eggs of *G. salinaris* may develop by parthenogenesis receives strong support from a study of sections of eggs laid by a female not associated with males. In Fig. 20 is shown a transverse section of one of her eggs and it can clearly be seen that development is well started. Twelve eggs out of the batch were sectioned, and it was found that eleven had started to develop, although apparently not in a normal manner. It is not improbable that some few eggs may develop normally and eventually produce larvæ. The question of parthenogenesis in the *Solidago* moths is one needing further study.

It might be worth while to add that parthenogenetic development among Lepidoptera is by no means unknown. DeGeer is given credit for having first discovered long ago that certain butterflies belonging to the genus *Solenobia* lay unfertilized eggs which develop into normal imagines, and later von Siebold not only confirmed this observation, but also discovered that *Psyche helix* reproduced parthenogenetically. It has since been shown by several workers that the silk moth, *Bombyx mori*, may under certain conditions reproduce by parthenogenesis.

III. Parasites of Gnorimoschema salinaris.

Riley reports six hymenopterous parasites for *Gnorimoschema gallæsolidaginis*, and in addition to these he found a beetle larva and another lepidopterous larva which intrude as inquilines within the cavity of the gall made by this species. At least five hymenopterous parasites have been found associated with *G. salinaris*. The most important of these is *Copidosoma gelechiæ*, which is by far the most common parasite attacking the moth. The other four species are *Calliephialtes notanda* Cress, *Epiurus* sp., *Eurytoma* sp. (pupa), and *Pseudacrias sexdentatus* Girault. The first of these four occurs most frequently, while the last has been observed but a few times. However, it is of special interest, inasmuch as it is the only observed case of a second parasite emerging along with *Copidosoma*, although the larvæ of other species have been found associated with the larvæ of *Copidosoma*. On September 3, 1914, six individuals, all females, emerged together with a brood of about one hundred Copidosomas from a single carcass. The small pupæ of *Pseudacrias* lying among those of *Copidosoma* were observed through the transparent chitin of the carcass of the host some days prior to their emergence. They were not grouped together but scattered about in different parts of the carcass. Each pupa was inclosed in a chamber very much smaller than, but exactly similar to that containing a *Copidosoma* pupa.

Usually *Pseudacrias* larvæ do not pupate until after the larval host has undergone this process. About a dozen *Gnorimoschema* pupæ have been found containing *Pseudacrias* pupæ, which later emerged. It is not probable that *Pseudacrias* is polyembryonic. First, because both male and female individuals usually emerge from the same pupal host; and second, because the individuals do not come out at the same time. The single instance of six females ssuing simultaneously with the brood of *Copidosoma* can be explained by assuming that a single female deposited six fertilized eggs in the host at the same time. However, this case is of special interest as it demonstrates the synchronous development in a single host of the broods of two distinct parasites, and thus supports Wheeler's ('10) suggested explanation of Silvestri's so-called asexual larvæ in *Litomastix*.

In addition to the five hymenopterous parasites, there are two insect larvæ associated with the larva of *G. salinaris*. They are undoubtedly inquilines. One of these is a beetle and the other a lepidopterous larva (Fig. 5). Judging from Riley's account, these two species are very similar to if not identical with the corresponding inquilines reported by him for the galls of *G. gallæsolidaginis*.

IV. Development of Copidosoma gelechiæ.

1. *The Polygerm Stages.*

(*a*) *Youngest Stages.*—We have not secured the cleavage stages of *Copidosoma*, owing to the fact that they occur earlier in the year than we have been able to reach Woods Hole. Therefore, in describing the developmental processes which have their inception in the cleavage stages, we must rely upon the work of other investigators in this field for our interpretation of the significance of these processes.

The youngest stages secured were found in a small larva of *Gnorimoschema*, taken June 21, 1913. The series of sections of this small caterpillar contains three young polygerms of *Copidosoma*. Evidently the egg from which the caterpillar developed had had three parasitic eggs deposited in it. Two of the polygerms, which lie close together, are situated in the first and second body segments of the larva, just beneath the hypodermis; while the third is found in sections 5 to 14 posterior to these, and is also situated just beneath the hypodermis of the host.

The three polygerms are not of the same size, as is indicated by the following measurements: Of the two specimens lying close together, the larger measures 150 μ by 82 μ and runs through 15 sections (150 μ), the smaller measures 103 μ by 71 μ, and is found in 12 sections; the single specimen measures 179 μ by 95 μ and occupies 8 sections only.

In structure the three polygerms are practically identical. Each consists of two distinct zones: (1) An outer relatively thick zone containing a large number of nuclei irregularly placed, and (2) a central region containing the embryonic nuclei (Fig. 19). In the absence of the earlier stages, it is not an easy matter to interpret the conditions seen in these polygerms. In the main they correspond most nearly to the conditions in the egg of *Litomastix* (*Copidosoma*) *truncatellus*, as described by Silvestri ('06). I therefore interpret the outer zone to be the product of the "polar oöplasm" plus the "polar nuclei," while the central region contains the true embryonic nuclei, derived from the fertilized nucleus, or in the case of parthenogenetic development, from the matured egg nucleus.

There is of course one essential difference in the corresponding stages of *Litomastix* and *Copidosoma*. In the polygerm of the former the central region is composed of a solid mass consisting of distinct cells, while in the latter this region is on the point of being broken into multi-nucleated masses, which form the primordia of the embryos (cf. Fig. 19 *A* with Silvestri's Fig. 33, Pl. III.). It may be that the embryonic nuclei are delimited by cell walls in *Copidosoma*, although one can not make them out with certainty, even under the highest powers of the microscope. Judging from the work of other investigators, one would expect to find the embryonic nuclei surrounded by cell walls. In *Ageniaspis*, Marchal ('04) first reported that the early embryonal masses were pluri-nuclear in character, but Silvestri ('08) and Martin ('14) have later demonstrated that the nuclei are surrounded by cell walls. In *Copidosoma* the embryonic nuclei are often so closely packed together that the demonstration of cell walls would be extremely difficult.

The three polygerms mentioned above are of particular interest, in that they show very clearly the manner in which the central mass with its nuclei breaks up to form the primordia of the multiple embryos. The central region itself consists of two distinct substances. (1) A granular protoplasm in which the embryonic nuclei lie, and (2) a more fluid-like material which becomes greatly shrunken during the process of fixation, and which in sections appears as a precipitated substance (Fig. 19 *A*, *P.M.*). As to the origin of these different substances we know nothing, but their subsequent history is clear. For the sake of clearness in description we shall use the following terms: (1) *Nucleated Membrane* for the outer zone; (2) *Granular Layer* for the protoplasm containing the embryonic nuclei; and (3) *Precipitated Material* for the shrunken fluid-like substance.

(*b*) *The Nucleated Membrane.*—In these young polygerms the outer zone stains more deeply than the central mass. The "polar nuclei" have no definite arrangement, but are irregularly scattered throughout the protoplasm. The entire zone therefore is in every sense of the word a syncytium. As the polygerm grows in size the nuclei become arranged into a single layer, and the protoplasm thins out, thus forming a true nucleated membrane about the central or embryonic portion of the egg (Fig. 21, *N.M.*). In the later history of the polygerm some of the nuclei are clearly surrounded by cell walls, that is, there is a tendency for the membrane to become cellular.

At first the young polygerms are naked, that is there are no elements from the host tissue laid down on the outer surface of the nucleated membrane. Later a few mesenchyme cells are found on the surface of the membrane, and still later these cells give rise to the adipose tissue (Fig. 22, *A.T.*), which may completely surround the polygerm.

(*c*) *Precipitated Material.*—This material occupies the central portion of the polygerm. Apparently it is formed through the action of the fixing reagent upon the fluid-like protoplasm. In sections it is very much shrunken, thus leaving an irregular clear space (Fig. 21, *C*). As we shall see later, it persists throughout the entire polygerm phase of development.

(*d*) *The Granular Protoplasm and the Embryonic Nuclei.*—In Fig. 19 the condition of the embryonic nuclei and their surrounding granular protoplasm is especially clear. Most of the nuclei are indifferently scattered in the protoplasm, but some of them are collecting into groups. The number of nuclei in each group is variable; some groups contain only two or three nuclei, while others may have as many as ten or twelve. The granular protoplasm surrounding a group of nuclei soon rounds off and the primordial embryo with its surrounding layer lies free within the more fluid contents of the central region of the egg (Fig. 19 *A*). The more usual condition is for the spherical mass to remain attached at one side to the peripheral layer of the granular protoplasm (Fig. 19 *B*, *P.E.*). Eventually all of the embryonic nuclei thus become included in these spherical masses of protoplasm, and thus become isolated as primordia of the embryos.

The condition at the close of the formation of the primordia is shown in Fig. 21. This specimen was found in a series of sections of a 3 mm. caterpillar, taken June 15, 1914. In the median section it measures 113 μ by 203 μ, and runs through 40 sections (280 μ). It lies in the middle portion of the body cavity, to one side of the intestine, which on account of the size of the polygerm is pushed out of place. As compared with the preceding polygerms this one is very much larger and shows a number of important changes. The nucleated membrane has become much thinner and its nuclei are arranged more or less into a single layer. The adipose tissue is being laid down on the outer surface of the membrane. The most important change, however, has occurred in the embryonic masses themselves. The protoplasm which surrounds a group of nuclei is differentiated into two distinct regions. The central part, crowded with nuclei, stains somewhat lighter than the peripheral zone, which forms a relatively dense layer about the central core (Fig. 21, *P.E.*). There are still a few nuclei which have not as yet been surrounded by the dense layer, but this stage marks approximately the end of the division of the germ into separate embryos.

(*e*) *Growth of the Polygerm and the Formation of the Primary Divisions or Masses.*—Upon the completion of the primitive embryos, the polygerm grows very rapidly. It first extends in the direction of its long axis, soon transforming into an elongated cylindrical structure. One specimen showing this condition measures in section 148 μ by 430 μ. It never becomes an elongated tube as does the polygerm of *Ageniaspis*. During this growth the adipose tissue is laid down in the form of a thick layer about the polygerm. One of the easiest ways in which to find a polygerm of this and later stages is to examine the large fat bodies lying in the middle region of the body cavity of the larval host. If the caterpillar is parasitized one of these bodies is almost certain to contain the polygerm.

After the elongated condition is attained, the further growth of the polygerm may take place in any direction. In some cases the extension is mainly in one plane, thus transforming the polygerm into a flat, plate-like structure (Fig. 13). In other cases it forms a thick irregular mass (Fig. 11), and when viewed as a whole mount shows many elevations on its surface, due to the breaking up of the entire polygerm into single masses, each of which contains an embryo.

During the rapid expansion of the polygerm a very important change takes place in its structure, whereby each embryo become enclosed in a double involucre. The first step in this process begins just prior to that period of development in which the polygerm attains its elongated, cylindrical shape. It consists in the formation of constrictions in the nucleated membrane which break up the single polygerm into a series of primary divisions or masses (Fig. 15). In the specimen shown in this figure there are about twelve of these masses. Each primary mass has the same general structure as the original single polygerm. It is surrounded by a portion of the nucleated membrane, contains precipitated material, and has a variable number of embryos, from five to fifteen or more.

In Fig. 22 one end of a longitudinal section of a polygerm is shown with the completed primary masses. Three of these masses are seen in the figure, together with a portion of a fourth. Attention should be called to the fact that the adipose tissue, although in contact with the polygerm, is still a distinct structure. In the process of forming the primary masses not all of the elements of the nucleated membrane are taken into these structures. Some of them are left behind and later lie in the inter-embryonal spaces or interstices. In Fig. 22 a number of these elements (cells and nuclei) are shown at the point marked "*N*," lying between the primary masses and the adipose layer.

In another portion of the same polygerm a single primary mass is being constricted off laterally. It appears as a bud extending from the main body of the polygerm. It is such cases as this which give rise to the condition frequently seen in whole mounts, in which the surface of the polygerm displays many protuberances.

(*f*) *Formation of the Secondary Masses.*—The primary masses soon become broken up into secondary masses. This is also brought about by constrictions of the nucleated membrane (Fig. 23). These secondary masses may contain more than one embryo, in which case they immediately form constrictions which result in producing still smaller masses, each of which contains a single embryo.

In the constrictions which lead to the cutting off of a single embryo with its involucres, some of the precipitated material is enclosed between that portion of the granular layer which is in contact with the embryo and that part lying adjacent to the inner surface of the nucleated membrane. These two parts of the granular layer then fuse, forming a single involucre in which are the spaces containing the precipitated material (Fig. 24). The embryo is thus surrounded by two involucres, a granular layer, and a nucleated membrane (Fig. 26). In some cases the precipitated material may be so abundant as to form a solid zone between the inner and outer parts of the granular layer; in others it is small in amount and gives the appearance of much flattened nuclei lying within this layer (Fig. 26, *P.M.*).

(*g*) *The Inter-embryonal Substance.*—At the close of the formation of the single embryonic masses and their involucres the inter-embryonal interstices are already filled with a substance derived from several different sources. It consists of a plasma-like matrix in which are embedded cells and nuclei. We have already noted that during the formation of the primary and secondary masses some of the elements from the nucleated membrane are not included in the outer involucre, but are left in the inter-embryonal spaces. During the early history of the inter-embryonal substance, it consists mainly of product from this membrane. Later cells from two other sources enter into its formation. First, leucocytes from the host are found embedded in the matrix. They are especially abundant in those regions of the polygerm exposed directly to the body cavity, that is near a surface barren of adipose tissue. Second, fat cells from the adipose layer invade the inter-embryonal spaces. The fat cells are the last elements to enter the inter-embryonal substance. In Fig. 13 a wedge-shaped mass of fat tissue is seen lying between the embryos in the middle region of the polygerm, on the upper side. Perhaps it would be more correct to say that the embryos bud out into the adipose tissue. Thus in Fig. 24 a single primary mass has been budded off into the adipose tissue.

The final condition of the polygerm at the end of the formation of the inter-embryonal substance is shown in Fig. 16. The adipose tissue has invaded the inter-embryonal substance from all sides of the polygerm and has become an organic part of this substance. The fat body and the included polygerm become an extremely complex structure, which may be called the *polygermal mass.*

2. *Dissociation of the Polygermal Mass.*

The setting free of the larval parasites into the body cavity of the host is brought about through the dissociation or disintegration of the inter-embryonal substance. The fat brought into close contact with the embryos by the invasion of the adipose tissue is digested and absorbed by them. It is therefore the first component of the inter-embryonal substance to disappear. That the fat is digested and consumed by the embryos is evidenced by the fact that the numerous other fat bodies remain intact during this period. The disappearance of the fat leaves the embryos loosely held together by the plasmalike matrix, which in turn soon disintegrates, freeing the larvæ.

The first larvæ to be set free are those situated at the periphery of the polygermal mass. Such larvæ are usually the largest present in the mass. As the inter-embryonal substance slowly disintegrates the remainder of the larvæ are gradually set free (Fig. 17). The earliest date at which free larvæ have been found was July 19; the latest, July 31. In the vast majority of cases the mass dissociates during the last week of July.

The larvæ retain the involucres for some time after being set free (Fig. 18). Once free in the body cavity they proceed to devour the contents of the host, first consuming the fat tissue, and finally the various organs. The last internal organ to disappear is the intestine.

3. *Pupation, and the Emergence of the Imagines.*

Pupation in *Copidosoma* occurs during the first ten days of August. The pupa stage lasts twenty-eight days. As stated above, the larvæ destroy all of the internal organs of the host, and consume such portions as are dissolved by the action of their salivary secretions. The undissolved portion consists largely of the chitinous parts of the tracheæ. The larvæ also destroy all of the body wall except the superficial layer of chitin. During the process of pupation the non-digested content of the caterpillar hardens and forms the thin-walled, oval chambers in which the parasitic larvæ lie and in which they undergo their transformation into pupæ. The superficial layer is perfectly transparent, and at first is very flexible. Later, as drying occurs, it shrinks in on the walls of chambers and becomes hard and rigid, the whole forming the typical mummified carcass (Figs. 2, 4, 6). Practically all of the pupæ are oriented in a definite fashion in the carcass. Their heads are directed toward the anterior end of the carcass. Just before becoming immobile, the *Gnorimoschema* larva almost invariably turns the head upward in the gall chamber; likewise, the parasitic larvæ, just before pupating, orient themselves so that their heads are directed upward, in the direction of the anterior end of the carcass.

The imagines come out during the last week of August and the first week of September (Table I.). They escape by gnawing holes through the walls of the chambers and the superficial chitinous layer, both of which become very fragile. As a rule they all emerge practically at the same time. Several cases have been observed in which the entire brood has escaped within a period of ten minutes.

Once free from the carcass, they immediately gnaw a hole through the wall of the gall. Their escape is greatly facilitated by the habit of the caterpillar, just before becoming immobile, of eating out a passage-way to, or nearly to the epidermis of the plant. But in no case does the parasitized caterpillar secrete a silken plug. Hence, in order to escape to the exterior, the parasites have only to cut through the remaining thin portion of the wall.

The parasites must winter over in the imago state; otherwise they would not be able to parasitize the normal or spring eggs of *Gnorimoschema.* Copulation, however, takes place immediately after the adults emerge, but the females do not parasitize the fall eggs of this moth. Only on one occasion has an attempt to ovipost in such eggs been observed. In this instance the few females which made the attempt were not able to penetrate the shell of the egg with the ovipositer.

TABLE II.

TABLE SHOWING VARIATION IN LENGTH OF LARVÆ IN THREE LOTS OF COPIDOSOMA.

Length in Lines.	Lots.			Length in Lines.	Lots.		
	I	II	III		I	II	III
1				29		3	3
2				30		10	7
3			2	31		3	1
4			3	32	1	5	
5			10	33		1	1
6		3	14	34		2	3
7			13	35		4	3
8		1	12	36		2	1
9			4	37		8	5
10		1	3	38		1	4
11		1	4	39	1	2	2
12			2	40	3	3	9
13			2	41	4	4	4
14			2	42	2	7	5
15	1		6	43	2	3	7
16	1		3	44	1	3	7
17	4	4	12	45	3	5	2
18		1	7	46	1	8	4
19	1	6	16	47	1	5	3
20		4	17	48	1	10	1
21			9	49	2	13	2
22		9	7	50		10	2
23		5	6	51		4	
24		3	4	52	2	8	
25		3	13	53		1	
26		3	5	54			
27		1	4	55	1	2	
28		4	1	56			

4. *The Abortive Embryos.*

One of the most interesting discoveries made in connection with the study of *Copidosoma* is what we shall call the abortive embryos or larvæ, to which brief reference has already been made. Abortive embryos occur in the development of many different species of both invertebrates and vertebrates. They are especially common in mammals. For example, my colleague, Dr. C. G. Hartman, has found a great mortality of embryos in the development of the opossum. Degenerating embryos are found throughout the brief but entire period of gestation. Abortive embryos have been found in at least three other species which have a polyembryonic type of development. One of the two embryos which develop from a single egg of the earthworm, *Lumbricus trapezoides*, sometimes degenerates. Fernandez ('09) has observed rudimentary embryos in the South American armadillo, *Tatusia hybrida*, and I have on several occasions seen them in the blastocyst of *Tatusia novemcincta*. But in no case with which we are acquainted is their number and constancy of occurrence so striking as in *Copidosoma*.

Our attention was first attracted to these abortive embryos while dissecting out a batch of larvæ from a large caterpillar. Most of the larvæ in the lot were large and about on the point of undergoing pupation, but in addition to these large individuals, there were a number of smaller ones. At first it was supposed that two distinct species of parasitic larvæ were present, or that we had a condition similar to that described by Silvestri for *Litomastix*, of sexual and asexual larvæ. It was noted, however, that the small larvæ had the same general structure as the larger individuals, except that they still possessed the two involucres typical of all of the younger larvæ of this species.

A study of serial sections of more than a hundred polygerms has completely demonstrated beyond any possibility of doubt that the small rudimentary embryos are derived from the same egg as larger normal larvæ, and consequently do not belong to a different species. The sections show that degenerating embryos are to be found in every stage of development of the polygerm, from the time of the formation of single embryos until the larvæ are set free into the body cavity of the host. In Fig. 24 is shown a degenerating embryo which has not yet been completely cut off from its fellow by the constriction of the nucleated membrane. Its nuclei have already completely disintegrated. In Fig. 26 is another embryo well on the way to complete disintegration. Finally Fig. 17, which is a portion of a polygermal mass about at the close of dissociation, contains at least four or five rudimentary embryos. They stain darker than the normal individuals.

The degeneration of embryos or larvæ does not cease immediately after the dissociation of the polygermal mass, but such embryos are found up until the beginning of pupation. About fifty lots of free larvæ have been dissected out of caterpillars, and

Table III.

Table Showing the Number of Parasites in Female Broods.

Brood.	No. of Individuals.	Brood.	No. of Individuals.
1	25	46	200
2	42	47	201
3	49	48	207
4	52	49	210
5	73	50	210
6	89	51	212
7	91	52	213
8	95	53	213
9	100	54	214
10	100	55	215
11	106	56	215
12	108	57	216
13	115	58	216
14	119	59	217
15	120	60	229
16	121	61	234
17	122	62	236
18	124	63	236
19	124	64	237
20	125	65	245
21	131	66	248
22	137	67	250
23	142	68	251
24	145	69	254
25	146	70	256
26	150	71	257
27	151	72	260
28	153	73	261
29	154	74	264
30	156	75	272
31	161	76	275
32	163	77	280
33	164	78	284
34	167	79	286
35	174	80	292
36	174	81	301
37	178	82	314
38	178	83	328
39	179	84	335
40	181	85	338
41	183	86	340
42	189	87	347
43	192	88	378
44	194	89	385
45	195	90	395

Total = 17,864.
Average = 198.48.

almost without exception degenerating individuals were found. During the early period of the free larval stage, any given lot will show great variation in the size of the larvæ. To show this, all of the individuals of three lots have been measured in the terms of lines on the eye-piece micrometer scale (Table II.). In Lot I. there were only thirty-two larvæ. All but six of these would have reached maturity. Lot II. contained 176 larvæ, but at least twenty of these were degenerating. Lot III. contained 257 larvæ, and probably more than a hundred of them would have degenerated eventually.

A series of sketches of these larvæ is shown in Fig. 25, *A* to *H*. The first four or five of these types would have developed to maturity, but such larvæ as those illustrated in *F* to *H* degenerate. The most common types of degenerating embryos are the small spherical or oval-shaped masses (*G*, *H*). In one extreme case the lot of embryos consisted of about thirty of these masses, together with only a single normal larva. Doubtless many other similar masses had already degenerated.

It is difficult to assign any definite cause to the degeneration of these embryos, although it probably has something to do with nutrition. In some cases it seems to be due to the fact that the division of the egg has been carried too far. Some of the primordia receive but few embryonic nuclei, and these are invariably the first to degenerate in the polygerm. In other cases the degeneration is apparently due to the lack of proper nutrition. Most of the polygerms are early surrounded by the thick layer of adipose tissue, upon which the early development of the embryos depends. But other polygerms are almost if not entirely barren of adipose cells, and it is an observed fact that the mortality of embryos in such cases is exceedingly high. In Fig. 14 one of these cases is shown. This polygerm, which is devoid of fat tissue, contains more than a hundred embryos, not more than thirty or thirty-five of which have developed normally.

V. NUMBER AND SEX OF COPIDOSOMA PARASITES FOUND IN GNORIMOSCHEMA.

The number of matured parasites developing in the *Gnorimoschema* larva has been determined in 162 cases. This has been done by removing the carcass from the gall chamber a short time before the emergence of the parasites, and enclosing it in a small vial. After all of the parasites have emerged they are killed by filling the vial with 80 per cent. alcohol, and then counted under a binocular microscope. This procedure has the advantage of eliminating the possibility of contamination from other polyembryonic broods. Furthermore, the use of the binocular in counting enables one to distinguish readily the two sexes. The strong sexual dimorphism in *Copidosoma* makes this task rather easy. The females have the enlarged club-shaped, terminal segment of the antenna, and bright yellow legs, while the males do not have the enlarged segment and the legs are of a

Table IV.

Table Showing the Number of Parasites in Male Broods.

Brood.	No. of Individuals.	Brood.	No. of Individuals.
1	41	32	178
2	53	33	179
3	61	34	180
4	67	35	180
5	90	36	180
6	93	37	182
7	96	38	190
8	100	39	190
9	101	40	192
10	106	41	199
11	107	42	199
12	113	43	202
13	118	44	204
14	119	45	214
15	124	46	215
16	124	47	218
17	124	48	223
18	127	49	225
19	128	50	232
20	136	51	233
21	137	52	236
22	138	53	236
23	139	54	245
24	142	55	247
25	147	56	272
26	152	57	277
27	168	58	278
28	171	59	323
29	172	60	324
30	177	61	328
31	177	62	345

Total = 19,874.
Average = 175.32.

dark, more or less mottled color. One can therefore readily detect a mixed brood under the microscope.

The 162 broods studied were taken at random from the field, and therefore in all probability the data on numbers and sex yielded by them represent the approximate sex ratio for the species. These 162 broods contained a total of 31,001 individuals, or an average of over 191 to the brood. Ninety of these, or 55.56 per cent., contained only female parasites, 62, or 38.27 per cent., contained only male parasites, and 10, or 6.17 per cent., contained mixed broods of males and females.

There are therefore not only a larger number of female broods than male, but the average number of individuals in the former exceed that of the latter. Female broods average a little over 198 individuals to the brood (Table III.), while male broods average only about 175 (Table IV.). The range in the number of individuals in these broods (from 25 to 395 in the female, and from 41 to 345 in the male) makes it evident that these averages are of little significance, except, perhaps, to show that the fertilized egg is slightly more prolific than the unfertilized egg.

Of the total number of individuals (31,001), 63.41 per cent. are females and 36.59 per cent. males; but obviously the true sex ratio can not be based on these figures. It must be determined from the number of male and female broods. It would not be a difficult matter to determine this ratio were it not for the uncertainty of the origin of some broods. There is always the possibility in these insects that more than one parasitic egg has been laid in the egg of the host, and hence the parasites which later emerge may not constitute a true polyembryonic brood, but in fact represent two or even more such broods. Under the circumstances, the best that one can do is to determine approximately the sex ratio for the species. This can be done in the following manner. If we assume, as all previous workers have done, that each of the mixed broods is the product of at least two eggs, then, in accordance with the law of probability, we can determine the number of unmixed male and female broods, each of which must also have been produced from two eggs. Worked out on this basis, it is found that the ratio of females to males is 106/76 or a sex ratio of approximately 3 : 2

This leads to a discussion of mixed broods, and especially to a consideration of the question as to how such broods have come into existence. The obvious explanation of their origin is the one given above, viz., that they arise from two eggs. Marchal and Silvestri, who have studied the development of polyembryonic insects, both offer this explanation. They support the conclusion by citing the fact that two (or more) parasitic eggs are sometimes laid in the egg of the host. According to Marchal, such eggs develop independently, each producing a distinct polygerm and consequently a distinct brood. If the two eggs are of the same sex potentiality, the individuals developing from them will be either all females or all males, according to whether or not the eggs are fertilized or unfertilized. The dual origin of these double broods naturally elude detection in lots that have emerged. But if one of the two eggs is unfertilized and the other fertilized, the result will be a mixed brood, consisting of males and females. This conclusion of Marchal and Silvestri is strongly supported by the facts of polyembryonic dévelopment in the armadillos, in which it has been conclusively demonstrated (Fernandez, '09, Patterson, '13) that all of the embryos of a given pregnancy are the product of a single egg. As a result, mixed litters are never found in these mammals.

That mixed broods may arise from two eggs in *Copidosoma* is supported by the fact that two polygerms are sometimes found in a single *Gnorimoschema* larva. However, certain facts concerning the condition of mixed broods in this species, make it doubtful whether the origin of all such broods can be explained in this obvious way. Careful dissections of something over a hundred parasitized *Gnorimoschema* larvæ have revealed only two cases in which a single larva contained more than one polygerm. Since 6.17 per cent. of all broods are mixed, and since a similar number of unmixed broods would have a dual origin, we should expect to find over 12 per cent. of all parasitized larvæ containing two polygerms, but instead, less than 2 per cent. are found.

Another line of evidence which is not in harmony with the view that mixed broods are always the product of two or more eggs, is the great preponderance of females in certain lots. Of the nine complete lots (Broods 2 to 10) listed in Table V., the number of females in each case is greater than the number of males. In some cases (Broods 3, 4, 5, 7, 8), this difference is not so great but that the origin of each lot can be explained on the assumption that two eggs have been deposited in the egg of the host. But in Broods 2, 6, 9, and 10 the number of females in excess of males is indeed striking, making it difficult to explain the origin of such broods on the basis of two eggs.

In view of these facts, the writer is convinced that some other explanation must be offered for the origin of certain mixed broods; in fact, one involving the idea that a single fertilized egg may give rise to a few males as well as a relatively large number of females. This would be possible on the basis of the following assumption.

TABLE V.

TABLE SHOWING THE NUMBER OF PARASITES IN MIXED BROODS.

Brood.	No. of Individuals.	Females.	Males.
1*	89	20	69
2	162	153	9
3	172	92	80
4	207	126	81
5	216	176	40
6	235	223	12
7	241	161	80
8	300	235	65
9	304	292	12
10	337	316	21
Totals........	2,263	1,794	469
Average......	226.3	179.4	46.9

* This brood is not complete, owing to the fact that some of the larvæ and pupæ had been destroyed by a large dipterous larva.

If *Copidosoma* conforms to the general scheme for sex determination in insects, the females must have the 2 X chromosomes, and males the single X chromosome. Ordinarily, during the process of cleavage, all of the chromosomes in the fertilized egg divide equally, so that all of the nuclei entering into the formation of the embryos will carry the XX chromosomes, thus producing a brood of females. But if during the early development of the egg it should happen that the two X chromosomes in one or more cleavages should not divide but separate, one going to each pole of the spindle, each daughter nucleus would then receive a single

X chromosome. If such nuclei later divided in the typical manner and gave rise to embryos, such embryos would be males. One is encouraged to make this suggested explanation in the light of Bridges' ('13) discovery of the non-disjunction of the sex chromosomes in *Drosophila.* In *Copidosoma* the separation of the sex chromosomes during cleavage would be a case of "somatic" or "cleavage disjunction," while in *Drosophila* these chromosomes fail to separate or "disjoin" in the reduction division of the egg.

In conclusion attention should be directed to the frequency of *Copidosoma* in nature. At Woods Hole about twenty per cent. of all *Gnorimoschema* larvæ are infected with this parasite (Table VI.). As may be seen from the table, the extent of infection varies greatly in the lots of galls taken from different regions (those collected on a given date are all from a single locality). Plants which grow in exposed places, as along the

TABLE VI.

TABLE SHOWING PERCENTAGE OF PARASITIZED CATERPILLARS IN THE GALLS OF SOLIDAGO SEMPERVIRENS.

Number of Galls.	Date.	Parasitized by *Copidosoma.*	Normal Galls	Empty.	Parasitized by Other Parasites.
9	7-29-12	7	2	0	0
33	8- 5-12	5	15	0	13
33	8-17-12	9	16	5	3
56	8- 8-12	7	26	10	13
29	8-12-12	8	16	0	0
141	8-25-12	20	56	33	32
14	7- 7-13	1	13	0	0
16	7-14-13	0	13	0	0
39	7-15-13	8	31	0	0
38	7-19-13	4	*	*	*
23	7-23-13	2	20	0	1
38	7-26-13	6	*	*	*
27	8- 5-13	4	*	*	*
24	8-25-13	4	17	3	0
18	6-15-14	2	14	2	0
19	6-18-14	3	16	0	0
43	6-22-14	19	19	3	2
40	6-24-14	9	20	10	1
20	7-16-14	1	19	0	0
24	7-30-14	0	21	0	3
25	7-23-15	3	12	7	3
18	7-26-15	5	11	0	2
66	7-30-15	25	37	2	2
35	8- 4-15	14	35	1	6
Totals. . 828		166			

* Record incomplete. About 20 per cent. of the caterpillars are parasitized by *Copidosoma.*

roadside or barren spots, carry a higher percentage of galls than do those which are located in protected regions. Likewise, the moth larvæ from the galls of the former are more highly parasitized.

SUMMARY.

1. *Copidosoma gelechiae*, which is a parasite in the Solidago Gall Moth, *Gnorimoschema salinaris*, has but one generation a year.

2. The egg of this parasite is probably laid during the month of May.

3. The type of development in *Copidosoma* is polyembryonic. The number of individuals average about 191 per brood.

4. In the youngest stages secured the process of division of the egg into embryonic primordia is already in progress. The young polygerm consists of two distinct regions: (1) An outer zone, or nucleated membrane, containing the free polar nuclei; (2) a central region, containing the true embryonic nuclei.

5. The embryonic nuclei segregate into groups, which become surrounded by a dense layer of granular protoplasm and form the primordia of the multiple embryos.

6. During early growth the polygerm elongates into a cylindrical-shaped structure, which becomes broken up into several spherical, primary masses by the formation of constrictions in the nucleated membrane. Each primary mass receives several of the primitive embryos.

7. The primary masses become broken up into secondary masses by further constrictions of the nucleated membrane. At the end of these divisions, each embryo is separated from the others and is surrounded by an inner and an outer involucre—the former derived from the granular protoplasm and the latter from a portion of the nucleated membrane.

8. The interstices between these masses become filled with an inter-embryonal substance derived from at least three sources: elements from the nucleated membrane, leucocytes, and cells from the adipose tissue, which usually is laid down in the form of a thick layer on the outer surface of the polygerm. The entire structure thus becomes a complex, which may be called the polygermal mass.

9. The dissociation of the inter-embryonal substance sets the larvæ free in the body cavity of the host. This occurs during the last week of July.

10. Abortive or degenerating embryos are found throughout the entire period covered by the polygerm and free larval stages.

11. The free larvæ destroy the entire contents of the caterpillar, except the chitinous parts of the trachae, and leave only the superficial layer of chitin of the body wall intact. The detritus left in the larval chitin hardens to form thin-walled, oval chambers in which the larvæ lie and undergo pupation The superficial layer of chitin also hardens, and the larval skin thus becomes transformed into the typical mummified carcass, filled with the parasitic pupae.

13. Pupation takes place during the first ten days of August and lasts about a month.

14. The number of adult parasites emerging from the carcasses varies from 25 to 395. There is a preponderance of females, about 55 per cent. of all broods being females. Furthermore, the average number of females emerging from a single carcass is 198 as compared with 175 for the males. Ten mixed broods of males and females have been obtained. Some of these have doubtless arisen from two or more eggs; but it is suggested that such broods may also arise from a single fertilized egg, by a process of disjunction of the sex chromosomes during the early cleavage stages.

Woods Hole, Mass.,
August 12, 1915.

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DESCRIPTION OF PLATES.

PLATE I.

FIG. 1. A typical gall of *Gnorimoschema salinaris*, Busck, situated at the base of the stalk of the swamp goldenrod, *Solidago sempervirens*. × ¼.

FIG. 2. Gall cut open to show the position of the mummified carcass of *Gnorimoschema*. Natural size.

FIG. 3. Gall cut open and carcass removed to show the shape of cavity. Note that the walls of the cavity are smooth and that the excrement from the caterpillar is packed in the bottom of the cavity. Natural size.

FIG. 4. Mummified carcass from gall shown in Fig. 3. Natural size.

FIG. 5. Lepidopterous larva which is an inquiline in the gall of *Gnorimoschema*. Note the irregular shape of the cavity which contains scattered trash and excrement. Natural size.

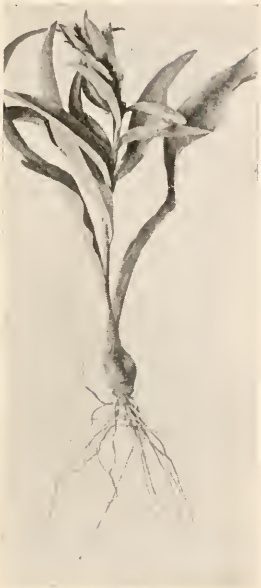
FIG. 6. This gall shows an incomplete passage-way, lying just above the head of the carcass. Normal size.

FIG. 7. Side view of a gall showing the orifice of the passage-way, closed by silken plug. Natural size.

FIG. 8. Stalk of swamp goldenrod containing two galls. × ¼.

FIG. 9. Gall containing a non-parasitized caterpillar. Natural size.

FIG. 10. Gall containing a parasitized caterpillar. Natural size.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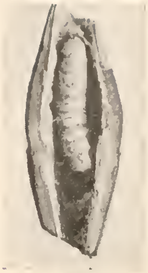
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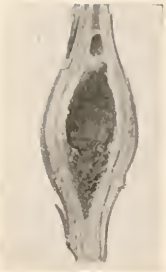
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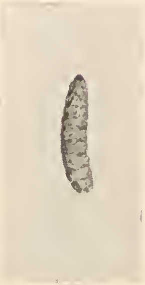
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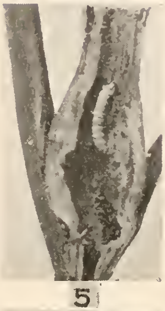
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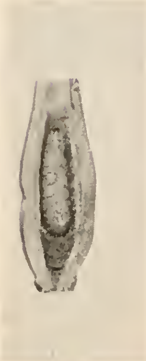
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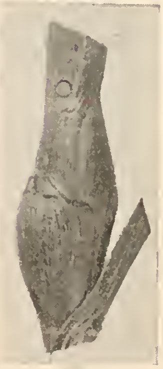
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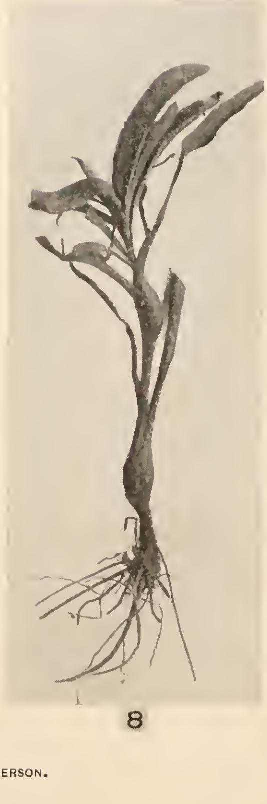
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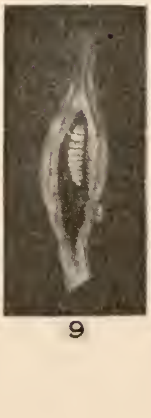
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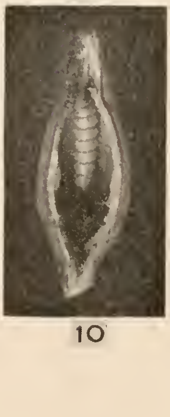
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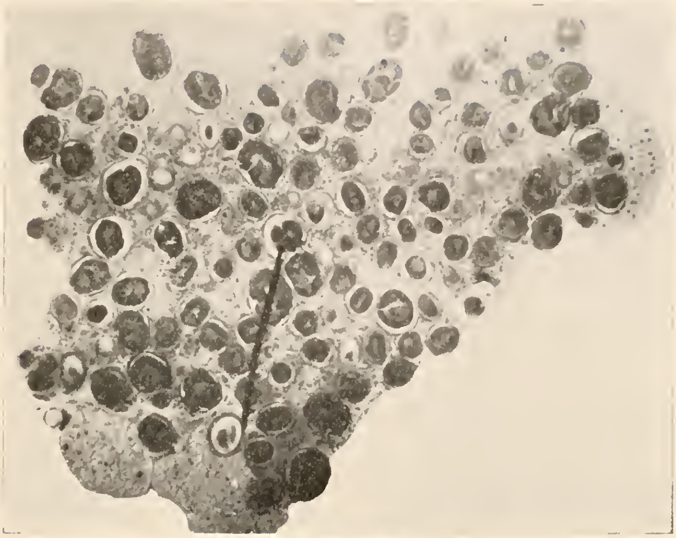
PLATE II.

FIG. 11. Photomicrograph of a section of an irregular polygermal mass. ×40.

FIG. 12. Photomicrograph of a single embryo from mass shown in next figure. ×180.

FIG. 13. Photomicrograph of a longitudinal section of a flat, plate-like polygermal mass. × 40.

FIG. 14. Photomicrograph of a spherical polygermal mass which is barren of adipose tissue. × 40.



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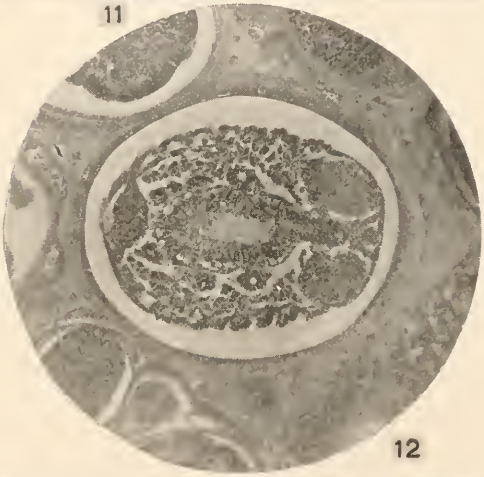

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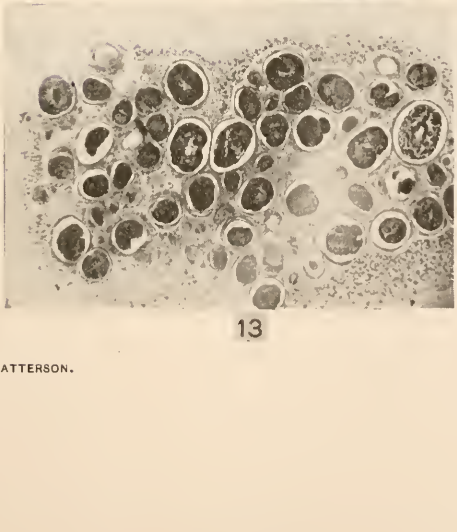

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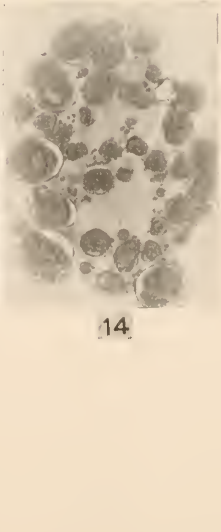

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PLATE III.

FIG. 15. Photomicrograph of the middle portion of longitudinal section of a small caterpillar. A fat body containing a polygerm lies just below the intestine. × 44.

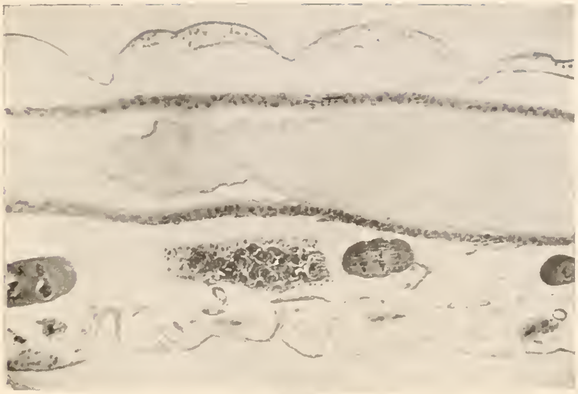
FIG. 16. Photomicrograph of a portion of a section of a polygermal mass which was about to begin disintegration. × 44.

FIG. 17. Photomicrograph of a section of a polygermal mass undergoing dissociation. × 44.

FIG. 18. Photomicrograph of a mass of free larvae from the body cavity of the caterpillar. Note that each embryo is still surrounded by the involucres. × 44.

Reference Letters Used in Plates IV.–VI.

A.E., Abortive Embryo.
A.T., Adipose Tissue.
C., Clear space left by contraction of Precipitated Material.
E.N., Embryonic Nuclei.
G.L., Granular Layer.
I.I., Inner Involucre.
I.S., Inter-embryonal Substance.
N.M., Nucleated Membrane.
O.I., Outer Involucre.
P.D., Primary Division of polygerm.
P.E., Primitive Embryo.
P.M., Precipitated Material.



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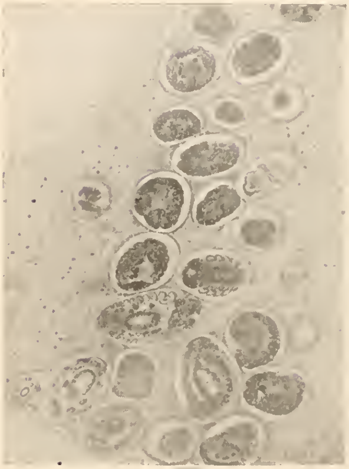

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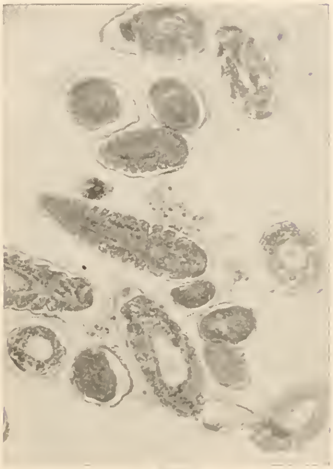

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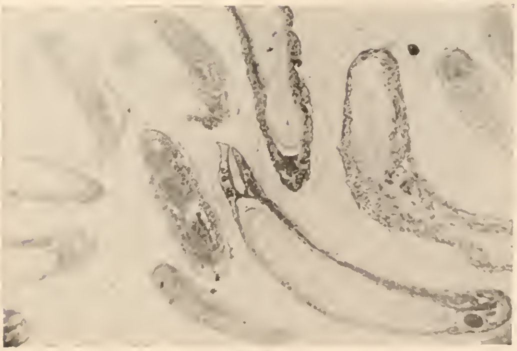

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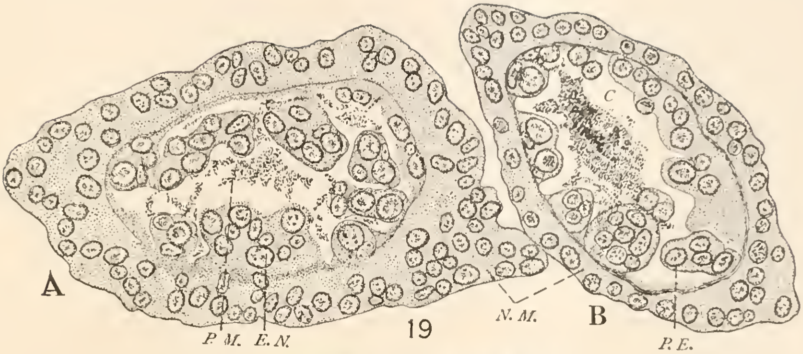
PLATE IV.

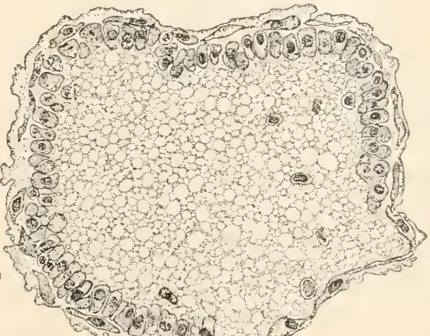
FIG. 19. *A* and *B* longitudinal sections of two polygerms lying close together in the same caterpillar. These polygerms show an early phase of the segregation of the embryonic nuclei to form the separate embryos. × 489.

FIG. 20. Section of an egg of *Gnorimoschema* which has started to develop parthenogenetically. × 173.

FIG. 21. Longitudinal section of a polygerm showing the end phase of embryo formation. × 480.


A
P. M.
E. N.
N. M.
B
C
P. E.

19



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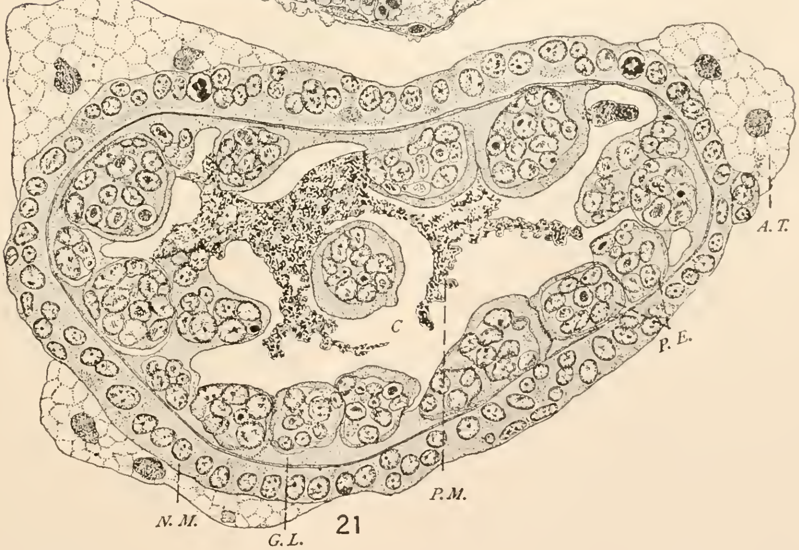
A. T.
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P. M.
N. M.
G. L.

21

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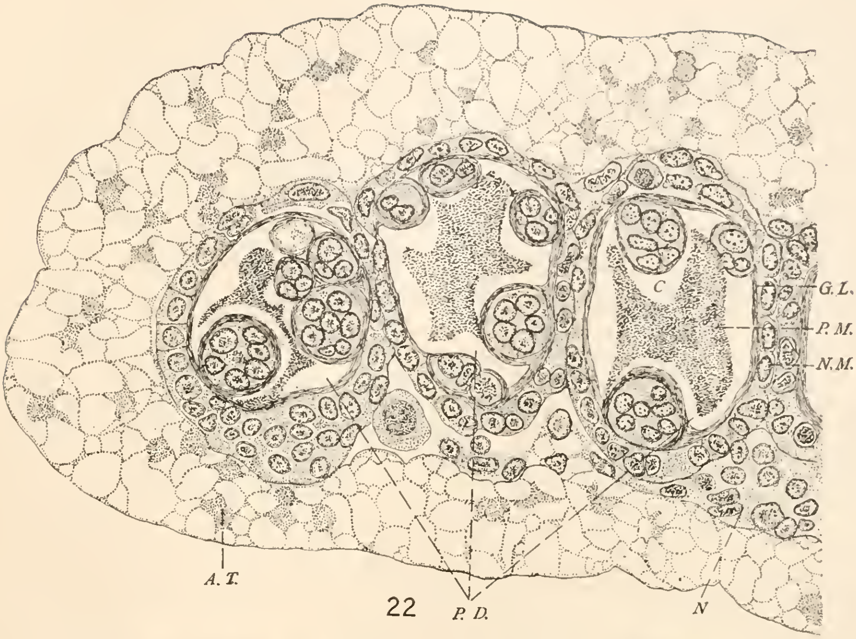
PLATE V.

FIG. 22. One end of a longitudinal section of a polygerm showing three of the twelve primary divisions into which it has been divided by constrictions of the nucleated membrane. × 373.

FIG. 23. Section of a primary mass showing the process by which it is further divided up into secondary masses by constrictions of the nucleated membrane. × 508.

FIG. 24. Section of a single isolated, primary mass about at the close of its division into single embryonic masses. × 257.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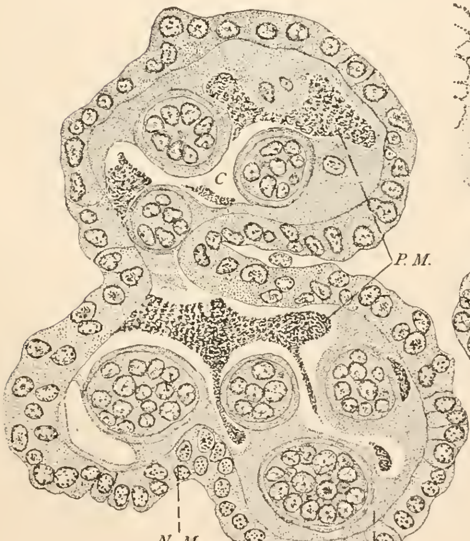

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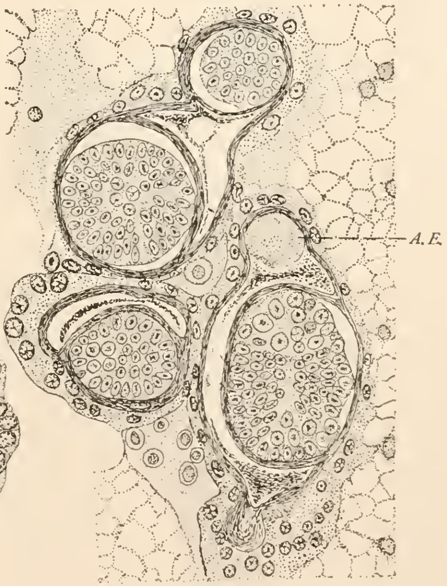

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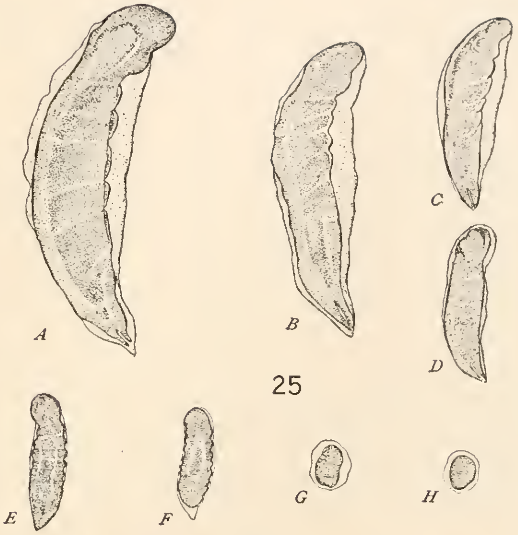
PLATE VI.

FIG. 25. *A* to *H*, Series of sketches from Lot III of the free larvae listed in Table III. This figure shows the great variation in size of the larvae from a single caterpillar. They are all drawn to the same scale.

FIG. 26. Detailed drawing of a section of one of the embryos seen in Fig. 13. It shows the relation of the inter-embryonal substance and involucres to the embryo. × 187.



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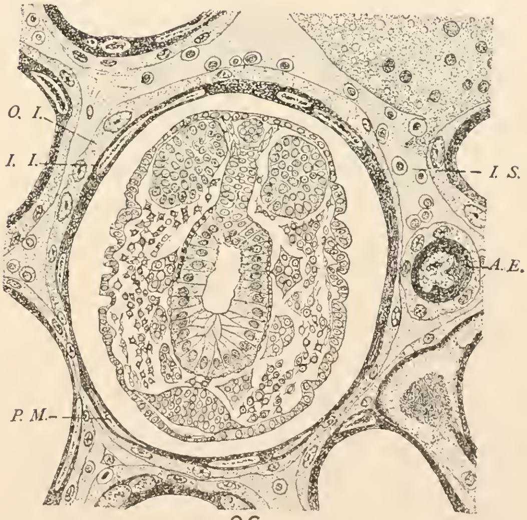
O. I.
I. I.
I. S.
A. E.
P. M.

26

DISTRIBUTION OF FOLLICULINA IN 1914.

E. A. ANDREWS.

The finding of vast hordes of the *Stentor*-like infusorian *Folliculina* both in 1912 and 1913 throughout the whole extent of the Severn River which is a brackish side branch of the Chesapeake Bay, led to further examination in 1914 to see if this were a phenomenon to be repeated annually or only a rare inroad of an outside fauna into new territory.

In 1913[1] *Folliculina* was found in inconceivable numbers living upon the leaves of the fresh water plants *Elodea* and *Potamogeton*, which have taken possession of definite zones of shallow brackish water along some fifty and more miles of extent of the river and its side creeks. It was also found on *Elodea* in Whitehall River, just to the north of the Severn.

In 1914 it was taken on *Elodea* from the head of the Magothy River, August 13, and on floating *Elodea* in the mouth of the Magothy, August 23, when it was also found living upon stunted *Elodea* growing in the narrow inlet canal to the nearly shut off side branch known as the Little Magothy. It was taken also at Deep Creek, a side branch of the Magothy.

As the Magothy opens into the Chesapeake some seven miles from the Severn, the distribution of Folliculina is quite extensive. Moreover, in 1880 Ryder[2] found *Folliculina* in great numbers upon oyster shells in shallow water on the west coast of the Chesapeake, and as he seems to have then been at St. Jerome, St. Mary's County, which is sixty miles down the Bay from the Severn, the distribution of *Folliculina* is known for side branches of the Bay opening into it seventy miles apart, approximately.

It is to be expected then that exceedingly large areas of the side waters of the Chesapeake may be inhabited by this little-known protozoan, which in the mid-summer season adds greatly to the plankton, or swimming fauna, as well as to the microscopic life attached to the summer vegetation of these waters.

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[1] See Biol. Bull., XXVI., No. 4, April, 1914.

[2] *Am. Nat.*, 14, 1880.

Its advent and departure in Chase's Creek, a branch of the Severn, showed in 1914 even more suddenness than in 1913, while its time of abundance was noticeably less though actual numbers present were even more vast.

Though searched for from the middle of June, every few days, *Folliculina* was found first on July 19, 1914. It then appeared only here and there, not on every plant of *Elodea* and on very few plants of *Potamogeton*. On the sprays of *Elodea* the *Folliculina* showed on comparatively few leaves, like black soot stuck on the leaves; both isolated individuals and aggregates occurred but there were very few large aggregates covering half the surface of a single leaf. Most leaves had none, some leaves had many scattered individuals. On the stems there were noticeable numbers of the small form of sac. The occurrence on leaves seemed entirely arbitrary as if from settlements of swimmers: the *Folliculina* was not now crowded toward the tips of the sprays but scattered along many inches of the spray.

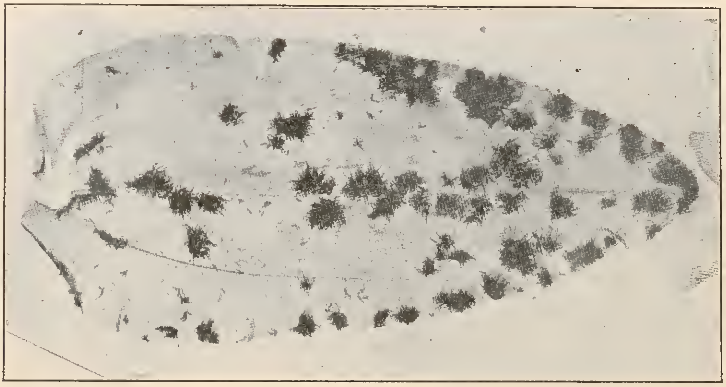

FIG. 1. Leaf of *Potamogeton* showing scattered colonies of *Folliculina*. × 3 diam. Photograph of preserved specimen.

At the date of this first appearance, jellyfish had been common for two weeks but the other conspicuous summer visitor to these waters, the young menhaden now for the first time came along the shores over the *Elodea*, which may be correlated with the feeding of the menhaden upon plankton in which the free swimming *Folliculina* may be included as possible food for the menhaden.

At this date the *Elodea* had grown up to a height of twenty inches and formed some flower stalks and buds at the surface, so that there had been a long period in which suitable attachment base for *Folliculina* was present but the *Folliculina* had been absent.

July 21 the water after long drought was turbid from the presence of plankton and the *Folliculina* had increased but little, appearing as black spots on one out of several hundred sprays of *Elodea* and one out of many thousands of *Potamogeton* sprays. Only a few of the leaves on each inhabited spray had dense aggregates, so that the question arises: why do the *Folliculina* crowd together in these rare, isolated aggregates? When sprays of these dates were put into aquaria they gave rise to free swimming forms, thus showing that these early settlers need not remain fixed but might contribute to additional distributions.

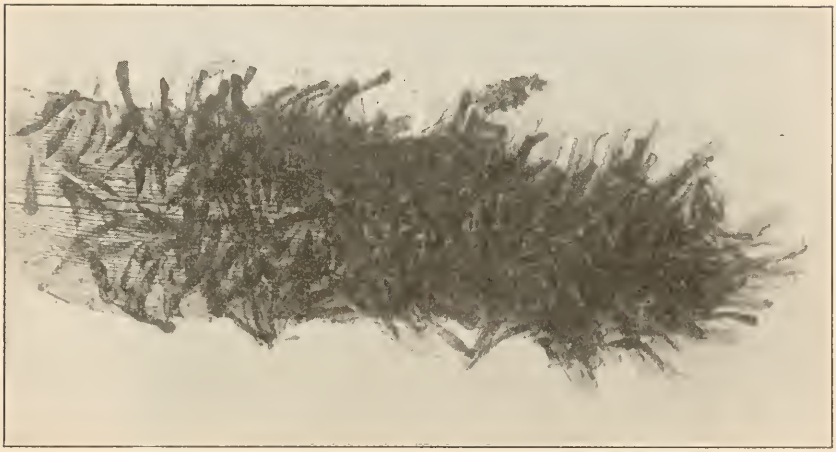

FIG. 2. Tip of leaf of *Elodea* covered with a colony of *Folliculina*. × 15 diam. Photograph of preserved specimen.

On July 27 *Folliculina* had become much more abundant upon sprays of *Elodea* and *Potamogeton;* some of the free-floating fragments on the surface appeared black with the accumulated

Folliculina. In the water also some free-swimming *Folliculina* could be seen near the surface swimming all through the water as well as close to floating plants.

Out in the Severn River a two-quart jar of water taken up at random at the surface showed several free-swimming *Folliculina;* three days later these had settled down on the side of the jar and were in two groups, two individuals in one and five in the other, so that at least seven were in the two quarts of surface water, which would make an immense number for the entire river.

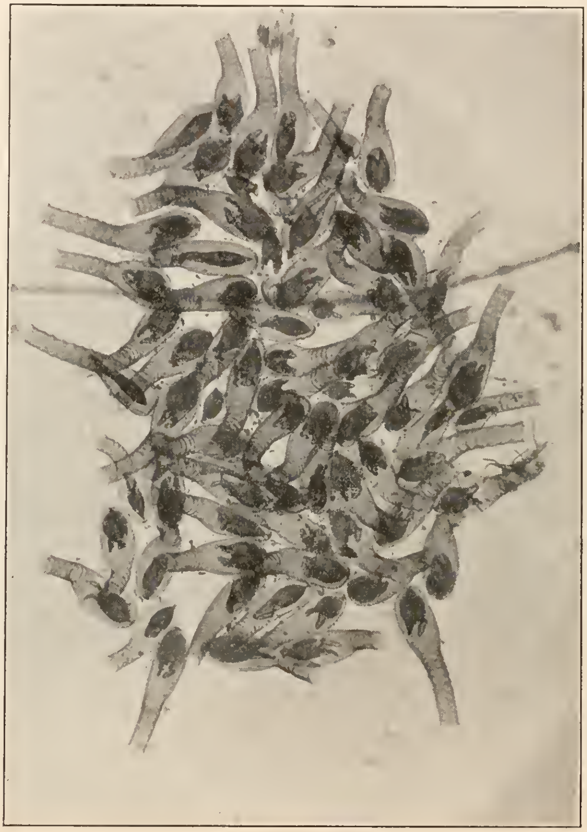

FIG. 3. Photograph of a preserved colony that had been formed on surface of the water in aquarium; showing form of case and tube spirals as well as animal rectracted within case. Enlarged 30 diameters.

By August 1 much of the *Elodea* growing in the *Elodea* zone along shore was black with aggregates of *Folliculina.* Free swimmers were in the water of the creek in vast numbers: a quart dipped from the surface at random showed in a white bowl from fourteen to one hundred, by actual count, for each quart of water from the surface. By drawing the bowl along the surface, the *Folliculina* swimming free were concentrated till thousands in a quart made it dark as if sprinkled with black pepper. Though these free-swimming Folliculinas easily escape notice in the greenish water turbid with plankton and sediment, they are readily observed in calm water by an eye near the surface; and standing in water five feet deep one may see them swimming rapidly in all directions, individually in straight and in curved paths. Many deep down in the water were seen best by holding a white object below them, but most of them were near the surface where they congregated especially about any floating object as fallen leaf or floating chip, seemingly influenced by its presence so that they swam toward it.

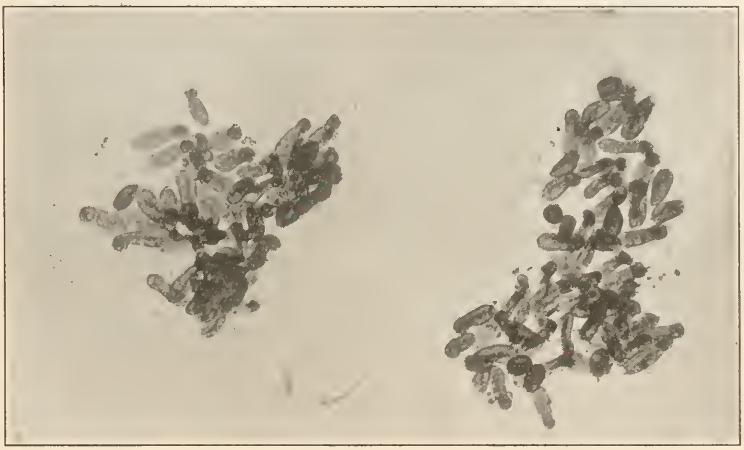

FIG. 4. Photograph of two young colonies of free swimmers that have just settled on surface of water in aquarium and formed sacs but no tubes: one individual on extreme left is still in motile form. Preserved specimen, × 20 diam.

While at this time the *Folliculina* continued to colonize the new growths at tip of the *Elodea* as fast as it grew so that the black aggregates crowded on the young leaves nearly to the tip where only the newest leaves were as yet unoccupied; by August 18 the extension of the *Folliculina* hosts had ceased. The tips of the growing *Elodea* were now bare or free from *Folliculina* back some twenty leaves from the tip and many of the old dwellings on the lower leaves were deserted. These dense black colonies on old leaves contained in fact but few living Folliculinas.

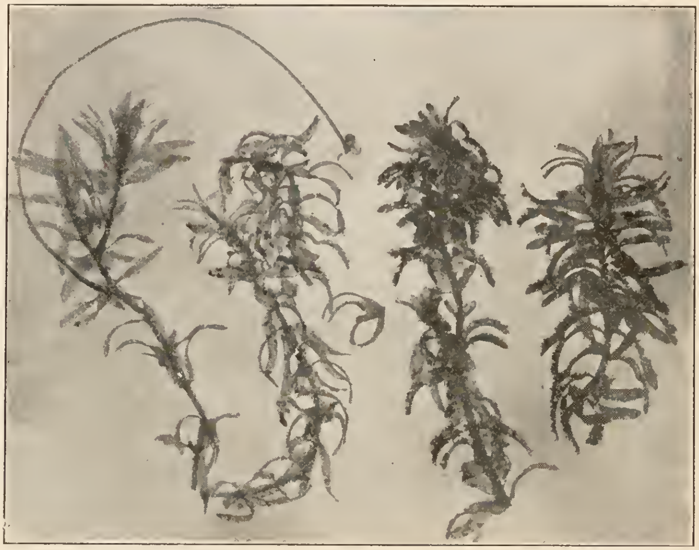

FIG. 5. Photograph of natural size sprays of *Elodea* preserved to show successive phases of colonization in 1914. Spray on left has grown enough to form flower but as yet but a very few isolated individual *Folliculina* have settled upon it. The next spray shows scattered tubes all along its length. The third spray shows dense aggregations of colonies even up to the tips of the rapidly unfolding new leaves. The fourth spray illustrates the subsidence in colonization: the new colonies no longer cover the leaves at the tip of the spray but these grow more rapidly than the new colonists occupy them and are left more nearly free from any Folliculinas.

By August 26 this falling off in the colonization and rapid disappearance of *Folliculina* was most pronounced: the *Elodea* sprays showed an abrupt transition from the lower leaves black from dense population of tubes, for the most part empty, to the upper leaves only sparsely inhabited with scattered individuals. Evidently some sudden change had operated not only to check the previously rapid spread of the Folliculinas onto new leaves but to almost exterminate them. Yet many remained alive here and there so that when large quantities of the *Elodea* were put into aquaria many free swimmers escaped. Yet these after forming new tubes on the surface of the water did not remain alive but had all vanished September 5, though in such apparently normal environment others had been kept two weeks in captivity earlier in the season.

Thus while appearing after the middle of July and being extraordinarily abundant in August, the *Folliculina* were all gone about the end of August and no way was found of keeping them longer. Their period of existence in accessible regions of the river was scarcely six weeks.

In 1913 they appeared before the end of June and a few lingered on to the first of September in nature and were kept in aquaria in a warm room till the 27th and a few till November 11.

In 1912 no live ones were found after September 8. This enormous crowding of the waters with free-swimming *Folliculina* and dense settlements of the case-making Folliculinas during about a month, the last weeks of July and the first of August, coincides with very high temperatures and abundance of microscopic plankton in these waters but it is not at all evident either why the Folliculinas should not come earlier, as they did in 1913, or remain later as they did in 1913 and 1912.

The great rapidity of their colonization of large areas suggests either very great immigration or else very rapid multiplication, or combination of both. As all material searched in the daytime in 1913 failed to show more than a few cases of multiplication, most all the free-swimming forms being merely the case-making forms again freed, material was collected at all times of the night in 1914, but here again but few cases of division were observed.

Hence it seems unlikely that fission of a few immigrants actually produced the vast numbers found on the leaves of plants, and it is probable that very large numbers came into the river suddenly from some outside source and these settling down, migrating out again, and in some cases increasing by fission, gave rise to the succession of dwellings covering the leaves for some two months.

The causes leading to the immigration as well as the causes of rather sudden diminution of numbers and utter disappearance remain entirely unknown.

The food of the case-inhabiting *Folliculina* being bacteria and some larger forms of plankton, the disappearance of *Folliculina* may well be associated with changes in food supply, in turn brought about in connection with such changes as those of temperature and salinity.

The motile forms take no food and may be enabled to settle and to continue migration and multiplication only when feeding conditions allow the sessile form to accumulate enough energy.

SUMMARY.

1. The vast swarms of swimming protozoans of the genus *Folliculina* that were found to settle down over the aquatic plants along the shores of side branches of the Chesapeake Bay in 1912 and 1913, came in even greater numbers in 1914, and it is therefore probable that this immigration and colonization is a regular annual phenomenon.

2. The incursions of swimming *Folliculina* do not take place as soon as the plants have grown enough to supply places for attachment, and the departure or disappearance of the living Folliculinas antedates the cessation of growth and final dying down of the plants upon which they settle.

3. As far as evidence is available the numbers that crowd the leaves arise more from immigration from without the area than from division of animals that have already settled in the area.

4. The times of appearance and disappearance differ in successive years.

5. It is suggested that conditions of food possibilities are determining factors in these inroads into the brackish fauna.

6. The great number of free swimming forms makes them, for the time being, an important factor in the plankton.

7. The crowding of the dwellings or cases on the leaves all along the shores is a considerable element in the transformation of matter which, arising from decay of organic materials, is transformed into bacteria and other plankton organisms, which in turn are eaten by *Folliculina* and enable them to secrete resisting tubes and sacs which finally settle to the bottom of the river.

PHENOMENA OF ORIENTATION EXHIBITED BY EPHEMERIDÆ.[1]

F. H. KRECKER.

It is a well-known fact that in alighting Ephemeridæ orient positively to a breeze. I became interested in this reaction and the observations made naturally lead to others on reactions to gravity and to light, and to the results of a conflict between any of these three stimuli.

The observations were made during the summer of 1915 at the Lake Laboratory of Ohio State University at Cedar Point on Lake Erie. Ephemeridæ appear here in almost incredible numbers. When a brood is at its height it is a very common occurrence to find piles of the insects three or four feet square and six to eight inches deep undenelectric lights. At a neighboring amusement resort several carts were required each morning to haul away the dead insects. The species with which the following observations are especially concerned is *Hexagenia variabilis*. The number, variety and arrangement of lights at the resort presented favorably conditions for observing the reactions to light of great numbers of individuals in what may be termed natural surroundings. The equipment used for experiments with air currents and gravity was simple and largely improvised. Nevertheless, since it is not primarily my purpose to measure intensity of stimuli or rapidity of reaction, I believe the results obtained have some interest and value.

REACTIONS TO A CURRENT OF AIR.

There was a question in my mind as to whether the positive orientation of the Ephemeridæ to a breeze is a response to the breeze per se or whether other factors are concerned. In order to test this I took a piece of glass tubing several inches long and sent through it a weak but steady current of air so directed as to strike the insects on the side of the body. They were resting on boards placed horizontally. A few of them flew away but most of them eventually faced the current. Individuals placed on a rough surface, such as a wire screen, which afforded a better foothold frequently tried to walk away. When facing the current of air an individual would raise its long, slender front pair of legs and extend them forward and upward at an angle of about 40 degrees. When held in this way the legs resemble antennae and it is possible they have a sensory function. However, cutting them off had no apparent effect on the reactions here in question. The time required for the turning reaction varied from an almost instantaneous response to two minutes. In the majority of cases the response was gradual and occupied from 30 seconds to one minute. The rapidity of reaction depended upon a correlation between the strength of the breeze and the part of the body it struck.

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[1] Contribution from the Department of Zoology and Entomology, Ohio State University, No. 43.

The influence of the area stimulated is shown in experiments with the wings. The latter are large in proportion to the body and meet over the back in a perpendicular position. They, therefore, present quite a broad surface. When a current of air of an intensity sufficient to blow the wings slightly to one side was directed against them individuals would react in fifteen to thirty seconds, whereas when this current was directed against the thorax or the abdomen the response was slower, if indeed any occurred. A stronger current directed against any of these parts brought about a correspondingly more rapid reaction.

In another series of experiments a current of air was directed from the posterior lengthwise of the body along the dorsal surface of a number of individuals. The response in these circumstances was also an eventual facing about to the current. A current of air striking an individual longitudinally along the mid-dorsal surface is neutral so far as lateral directions are concerned. In the cases here in question the current blew the wings to one side or the other and then as before the insects turned around toward the side on which the strain was exerted.

The experiments were repeated on a group of individuals from which the wings had been removed. The results from a current of air striking the insects on the side of the body were the same as before; the insects faced the current. However, when a current was directed from the rear longitudinally along the dorsal surface of the body the previous results were not repeated. In some cases the insects crawled with the current and away from the point of origin. In other cases they remained stationary and took an attitude similar to that assumed when facing the current. If the current became very strong they either attempted to crawl away or they retained the attitude until blown off their feet. When the current veered sufficiently to strike them on the side they began to turn toward it.

In these experiments with air currents the first noticeable response from the insects was an attempt to hold on to the surface upon which they were resting. This they did by fastening their claws firmly and even changing the position of the legs. When the current became so strong as to make it difficult to remain attached and especially when the body was blown over to one side the insects began to change position, rather hesitatingly it appeared, and to face about toward the direction from which the current came. When an insect reached a position where it did not seem to have difficulty in maintaining its hold it came to rest. This usually meant that it was directly facing the current, although sometimes it stopped at a point between a half and a complete about face. A half about face could generally be made complete by increasing the strength of the current.

When directly facing a current of air an individual is in the optimum position for resistance; it presents the least surface and the claws because of their backward curve have the maximum effect in holding the body. On the other hand when an individual stands sidewise to the current a greater surface is presented, the claws are not in a relatively favorable position and attachment is clearly more difficult. With regard to the more rapid reactions which result when the current strikes the wings it may be said that the proportionately great expanse of the wings above the body's center of gravity gives them such a leverage that the body is more easily tipped over, a strain is more quickly felt and attachment more quickly made difficult. In those cases in which a current struck wingless individuals from the posterior there was practically no obstruction to the current and it consequently did not so easily cause strain or seriously disturb the attachment and there was therefore no turning reaction.

It would appear from the foregoing experiments that the Ephemeridæ do not change position under the stimulus of a breeze until a strain is exerted on the organs of attachment. That this does not merely mean that the response was delayed, until a breeze of a given intensity developed is shown by the fact that a comparatively weak breeze directed against the wings alone had the same effect as was caused by a somewhat stronger breeze against the thorax. There is, therefore, evidence, I believe, for concluding that Ephemeridæ do not orient positively to a breeze because of sensations derived from the breeze per se but that they react positively to tension exerted on the muscles of attachment.

Reactions to Gravity.

The position of Ephemeridæ when resting upon a perpendicular surface is negative with regard to the earth's surface and usually approximately vertical to it, although variations as great as 45 degrees occur. On comparatively smooth surfaces the orientation is more generally an approximation to the vertical, whereas on surfaces such as a wire screen, which affords a good foothold at any angle, variations from the vertical may occur in 50 per cent. of the individuals concerned. Individuals picked up by the wings and replaced head downward, if they are not so disturbed as to fly away, will struggle to gain a foothold. The position of the claws, which are adapted to a vertical position, make attachment rather difficult. This difficulty is increased by the fact that the long abdomen is thrown forward and downward and thus tends to destroy equilibrium. On comparatively smooth surfaces such as a planed board the insects rarely succeeded in maintaining their equilibrium long enough to gain a footing. On a wire screen they were more often successful and once they gained a footing and their equilibrium they retained the new position. The picking up process caused so many of the insects to fly away that other methods were tried. Several individuals were placed in a vertical position on a straw hat held perpendicularly and then the hat was slowly revolved until the insects were upside down. The overhanging abdomen disturbed the equilibrium of some of them sufficiently to cause them to lose their hold and fly off. The others retained their footing, in some cases by changing the position of the legs, and remained in the inverted position for ten to fifteen minutes which was as long as they were watched.

In explanation of the position normally assumed on an upright surface the evidence derived from the experiments seems to indicate that the position taken is not a negative reaction to gravity per se but that it is largely, if not entirely, due to the character of the insect's means of attachment.

Results obtained from experiments performed to test the influence of a breeze upon the position of the insects on a perpendicular surface support this view. A current of air was directed against the side of individuals resting in the normal upright position on a perpendicular surface. As they turned the current was so directed as to bring them still further around. During the process some of them could not retain their foothold and flew off. The others turned completely around and faced directly downward. They maintained the inverted position at least as long as they were under observation, ten to fifteen minutes, which length of time, in view of a constant coming and going among those normally situated, seemed sufficient.

Reaction to Light.

The conclusions with regard to reactions of the Ephemeridæ to light are largely the result of observations made in the amusement resort already mentioned. The observations have to do mostly with artificial light. The insects react negatively to bright sunlight and seek the shade. They are strongly attracted to the lighter colors of artificial light. In the resort there are a great many electric lights of sixteen candle power intensity with colorless glass bulbs. Many of them are attached in a horizontal position to the sides of buildings in such a way that there is a perpendicular surface either above or below them and frequently on all sides.

The reaction to these lights seems to be satisfied if the insects can come to rest within a zone which begins approximately six inches from the light and covers a radius extending outward for about twenty-four to thirty inches. When individuals enter this optimum zone they alight, if a surface is available, and orient themselves in such a way that the body is parallel with a radius projecting from the light. After alighting the insects usually remain at rest, although there may be a certain amount of crawling toward a position nearer the center. This is more often done by those nearer the outer limits of the zone. When the insects are numerous they become arranged in rows consisting of individuals either directly behind one another or slightly to one side and they thus form a striking pattern of radiating lines.

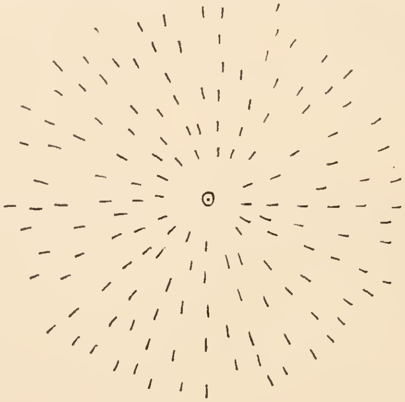

FIG. 1.

The accompanying figures illustrate the positions assumed with regard to lights in different positions and combinations.

The first figure illustrates the position assumed when the surface extends about a light in all directions whether the plane be horizontal or vertical. When any portion of the surface is absent the pattern is of course interrupted to a corresponding extent. The clear zone immediately surrounding the light was approximately six inches wide. I shall call it the excitement zone. Individuals that entered this zone became greatly excited and fluttered about the light in a confused state. There was no evidence to show that individuals at rest deliberately entered the excitement zone. Those immediately bordering on it were rather restless and occasionally in crawling about some were pushed into it and others on taking wing came within the influence of the light.

The second figure shows lights arranged along the lower edge of a perpendicular surface at intervals of twelve to fifteen inches. About each light was the usual excitement zone and upward from this extended the radiating lines of insects in the optimum zone. As shown in the diagram these lines were rarely at an angle of less than 35 degrees. This was due to the fact that below this point the lines from neighboring lights conflicted and caused such confusion among the insects as to obliterate regular alignment. The greatest confusion occurred in the comparatively short space between the lights where insects attempting to arrange themselves about one light constantly came into conflict with others attracted to the neighboring light.

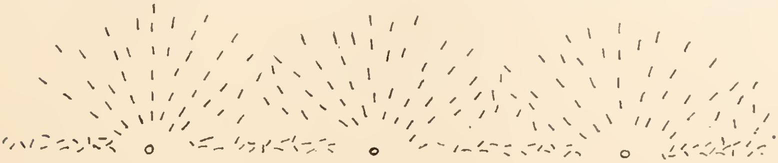

FIG. 2.

When the insects rested on a horizontal plane about a light they faced it. The most striking feature connected with the arrangement of the insects on a perpendicular surface was that the individuals on opposite sides of a horizontal plane passing through the center of a light had opposite ends of the body directed toward the light. The insects below the plane or parallel with it faced the light, whereas those that were above the plane were turned away from the light. In other words all the insects, except those parallel with the horizontal plane, approximated a vertical position with the anterior end uppermost. Those above the plane and with the posterior end directed toward the light were apparently as well content as those below the plane and facing the light.

The position of the insects on a horizontal surface shows that other things being equal they face the light. It is reasonable to conclude that their normal reaction to light is positive. The negative position assumed on a perpendicular surface above a light can be explained, in view of the air current and the inversion experiments, as being due to the difficulty experienced in maintaining a foothold in the inverted position.

Some observations were also made on the relative influence of white and colored lights. On the sides of one of the buildings in the resort there was a succession of alternating white, red and blue lights. The slightly yellowish white bulb attracted the insects in greatest numbers. There was the usual excitement zone and the regular alignment of those at rest. The number of insects about the red and the blue bulbs was decidedly small and as between the two lights about the same. These lights appeared to have a quieting effect on the insects. The alignment was similar to that described for white lights but there was no well-defined excitement zone, in fact the insects crawled about the bulbs without exhibiting markedly abnormal reactions.

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CELL MULTIPLICATION IN THE SUB-CUTICULA OF DILEPIS SCOLECINA.[1]

DALTON G. PAXMAN.

INTRODUCTION.

The process of cell division in cestodes as compared with that in other Metazoa is apparently quite abnormal. An examination of cestode material at once reveals the fact that mitotic figures are very rare, and that an explanation of the process of cell division analogous to any of the common types is apparently impossible. The opinion of the various workers in cestode cytology, as to how cell division is taking place, varies greatly. Some state that it occurs by mitosis, others by amitosis, while it has been asserted that nuclei arise 'de novo' from the cytoplasm.

Child ('07) noted the apparent infrequency or total absence of any evidence of mitosis in *Moniezia*, even in regions where rapid growth was taking place. He says, "If my observations are correct, amitosis is the more common method of division in the generative cycle, except during the period of maturation and early cleavage. And in the somatic cells of the adult body it appears to be the usual method at all times."

Young ('08), working with *Cysticercus pisiformis* describes what he calls the "de novo" formation of cells. He observed irregular masses of coarsely granular cytoplasm lying in the meshes of the parenchyma network. These masses contain numerous small deep staining granules scattered haphazard through the mass. Shortly succeeding the formation of these granules, a nuclear membrane is formed around them; the newly formed nucleus, together with a small mass of cytoplasm, becomes partly constricted from the parent mass; and the daughter cell has been formed."

Further, he says: "I believe that the nucleus in these forms is not a morphological, but a physiological entity; that the nuclear granules are fundamentally the same as the remaining protoplasm of the cell, but are differentiated therefrom under physiological conditions which we do not at present understand; that the granules are perhaps reserve material stored up in the nucleus for future use, the entire cell body being thus occasionally converted into a nucleus; and the nucleus varies in structure from time to time in response to the varing physiological demands made upon it. . . . Further if my interpretation of my observations be correct, then distinction between germ and somatic plasm is obviously impossible, a special vehicle for the transference of hereditary qualities is entirely wanting; such qualities must be transmitted by the undifferentiated protoplasm; cell lineage is manifestly lacking; a mosaic theory is plainly untenable; and the fate of any given embryonic element—whether it shall form parenchyma, muscle, nerve, etc.—must be determined by physiological causes alone."

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[1] A thesis presented to the graduate faculty of the University of North Dakota in partial fulfilment of the requirements for a master's degree.

Richards (1911), working with *Moniezia*, does not agree with Child. He says (p. 158): "I have after diligent search upon carefully prepared material been unable to establish a series of stages in the autoconstriction and subsequent division of the nucleus and cell body by amitosis. Considering the evidence as set forth, it seems to the writer that one is forced to the conclusion that mitosis is the method by which pre-oögonia and cleavage divisions are accomplished."

Mary T. Harman ('13, p. 223) states: "My observations have not shown that amitosis does not take place in *Taenia* or *Moniezia*, but they have shown no condition which cannot be as readily explained as the result of mitotic as of amitotic division."

Materials and Procedure.

The form I worked with was *Dilepis scolecina* parasitic in the small intestine of the double-crested cormorant (*Phalocrocorax dilophus*). These birds are found abundantly near the shores and on the islands of Devils Lake, North Dakota.

Immediately after the bird was killed, the cestodes were removed from the intestine and placed in fixing solution. Flemming's solution and cestode mixture were the fixatives used. Flemming's solution blackened the tissue so that the results from it were not satisfactory. The cestode mixture, however, gave excellent results.

The stains used were the following: Heidenhain's iron-alum-hæmatoxylin without counterstain; safranin counterstained with light green; thionin counterstained with acid fuchsin; methyl green counterstained with acid fuchsin; and safranin counterstained with water blue.

Observations.

I began my study of cell multiplication in cestodes without any previous knowledge of what had been done in the field of cestode cytology. Moreover, I completed the study of my material and drew my conclusions before I read any of the literature on the subject.

I have confined my study of cell multiplication in Dilepis to the sub-cuticula. In this tissue I have searched in vain for a single clear case of mitosis or amitosis. Moreover, in order to be certain I had not overlooked any, I counted 10,000 resting nuclei in the sub-cuticula of the neck regions of ten worms with the same result. Certainly active growth must have been taking place in this region, but it could not be accounted for by mitotic or amitotic division.

I have, however, observed numerous places in this region in which active cell multiplication was apparently taking place. Here multinucleate cells, such as shown in Fig. 1, have been observed. In addition to these, large protoplasmic masses were present, which varied in size from that of a single cell to that of perhaps fifty cells massed together. Fig. 2 shows a typical mass. These masses stain rather deeply with nuclear stains, and contain from one to five nuclei.

These masses are found abundantly in the neck region of every worm I examined, and occur, although less frequently, in the body region.

By reference to any of these figures it is seen at once that the mass of cytoplasm is out of proportion to the mass of the nuclei. Moreover, I have observed numerous lobes and occasionally even entire masses in which I was unable to find any trace of a distinct nucleus. Fig. 7 shows a lobe,[1] *i*, and Fig. 6 a mass of protoplasm, *h*, in which no well-defined nucleus is present. However, in this latter case the mass is so close to a nucleate mass that I cannot say positively that it is not continuous with it.

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[1] At focal levels other than that shown in the figure the lobe was seen to be continuous with nucleate masses.

By closely examining the nuclei present in these masses, I find that the nuclear membranes are very indistinct in many cases. Fig. 2 shows a mass in which the nuclei have indistinct membranes. Also one of the nuclei, *c*, has a somewhat less distinct membrane than the other, *b*. And this latter membrane is in turn less distinct than the membranes of the nuclei in the cell syncytium above it.

Moreover, a large number of nuclei have been seen which lack membranes completely. The nucleus consisted of a "nucleolus" or "karyosome" surrounded by a clear zone. Figs. 3, 4, and 5 show "karyosomes" which lack membranes. As Child and Young have already suggested, I believe this "nucleolus" represents the chromatin material of the nucleus.

By observing the protoplasm under high magnification (2,000 diameters) it is seen that the protoplasmic strands contain many dark staining granules of various sizes and shapes. Some of these granules were as large as the "nucleoli" of the complete nuclei; others, however, were so small as to be scarcely discernible. Fig. 4 shows a mass which contains a number of varying-sized granules. Fig. 5 shows a mass which contains a number of varying-sized granules one of which, *g*, is becoming surrounded by a clear zone.

The protoplasmic masses apparently arise by the outgrowth of protoplasm from certain cells of the syncytium. Figs. 2, 3, 4, and 6, show masses of protoplasm continuous with the syncytial cells around them. In Fig. 6, the developing mass is very small and contains no definite nucleus. In Figs. 2, 3, and 4, the masses are very large and contain from one to five complete nuclei. A large number of masses have been observed varying in size between these extremes. The nuclear membranes of the nuclei in the cells from which these masses are developing, contain very small, irregular granules which stain darkly like the granules in the cytoplasm. I have insufficient evidence for or against Young's view of the "de novo" origin of these granules. The chromatin granules may arise "de novo" in the cytoplasm and develop to complete nuclei in situ. Young bases his theory of the independent origin of granules from a cytogenic protoplasmic mass upon the following facts:

1. The occurrence of masses of granular protoplasm lacking any evident nuclei.

2. The occurrence of isolated "nucleoli" of varying size from ¼ to 1 micron in diameter, which are usually found in the above mentioned masses of protoplasm but occasionally lie free in the parenchyma strands.

I believe, however, that these facts may be equally well accounted for by assuming the extrusion of chromidia from a mother nucleus. Masses of granular protoplasm without any evident nuclei, which occur but rarely may be explained as having been severed from parent masses after impregnation with chromidia. The occurrence of isolated "nucleoli" can be accounted for just as well by assuming the migration of chromidia from the nuclei along the strands of the cytoplasmic network, as by the assumption of their development from the protoplasm in situ.

Young, in a later paper ('13) dealing with gametogenesis, in *Tænia pisiformis* says (p. 375): "I believe that new nuclei arise either from chromidial extrusions from old nuclei, or 'de novo' in the cytoplasm. . . . The structure of the nucleus—a loose collection of chromatin bodies without a membrane—renders the extrusion of chromidia an easy matter. After their extrusion new chromatin is added and that part of the cell containing them is constricted off, to give rise in its turn to other cells. . . . It is obviously impossible to say, however, whether any chromatin granule in the cytoplasm is a chromidial extrusion or a 'de novo' formation."

Since I have seen these very small granules, all of about the same size, present in the nuclear membrane as though impeded by it in their exit, along the strands of the protoplasmic network, from the nucleus to the cytoplasm, I believe that these granules are extruded from the mother nucleus. Moreover, since I have observed granules of various shapes and sizes, many of the larger ones appearing to be composed of three or four smaller ones partly united, and since I have often seen a number of granules clustered together, I believe that the larger granules are the result of the union of many smaller ones. Thus, I believe that the small particles of chromatin or "chromidia" are extruded from the mother nucleus. Then these "chromidia" unite here and there throughout the protoplasm to form larger granules or "karyosomes" which become surrounded by a clear zone. Finally the nuclear membrane is formed, producing a daughter nucleus. When a number of nuclei have been formed multinucleate cells are the result. Since the tissue is always a cell syncytium, constrictions of the cytoplasm around a nucleus finish the production of a daughter cell. Thus one mother cell may produce a large number of daughter cells.

Comparison with Tænia pisiformis.

In order to compare the process of cell multiplication in *Dilepis* with that in other cestodes, Dr. Young has permitted me to examine his slides of *Tænia pisiformis*, and *Cysticercus pisiformis*. Here I have identified the protoplasmic masses in both the adult and the larva. These also contain nuclei in the various stages of formation from chromidia to complete nuclei. The young larvæ show large numbers of protoplasmic masses developing in the cell syncytium. In the older larvæ the masses often show four or five nuclei developing membranes at the same time.

Discussion.

Cell multiplication by means of protoplasmic masses and the development of nuclei from chromidia, has, so far as I am aware, never been observed heretofore in Metazoa by anyone except Young. He has described the process as it occurs in *Cysticercus pisiformis* (Young, '08) and has noted it in some other cestodes (Young, '10) although his interpretation varies slightly from my own. I have, in the present paper given an account of it as it occurs in the sub-cuticula of *Dilepis scolecina*. It is true that chromidia have been observed in certain Metazoa, but no account of their functioning in the reproduction of the cell has ever been given previous to Young's paper on the "Histogenesis of *Cysticercus pisiformis*."

If cells are actually developing from protoplasmic masses in the manner described, we have here an exceptional method of cell multiplication, unlike anything previously described in Metazoa.[1] Moreover, if future research supports this view, the present theories of the role of the nucleus in heredity will have to be greatly modified at least with respect to cestodes.

As Young has previously suggested, the explanation of such a method of cell multiplication as this may rest on the fact that the cestode is highly degenerate in most characteristics due to its long period of parasitism. In the development of cells from protoplasmic masses the nucleus passes through a cycle in which occur stages resembling nuclei of lower forms. The protoplasmic mass with its diffused nuclei in the form of chromidia is comparable to a cell of the Bacteria or of the Myxophyceæ. In certain Protozoa also, as noted by many observers, the nuclear material at certain periods diffuses throughout the cytoplasm in the form of chromidia which may give origin to secondary nuclei, and these in turn to gametes. It is possible that the cestode nucleus has lost the power of mitotic division, accompanying the somatic degeneration of the worm due to parasitism. Richards, Harman, and others have shown, however, that we still find cell division taking place by mitosis in the sex cells and developing embryos.

Conclusions.

I have made the following conclusions in regard to cell multiplication in the sub-cuticula of *Dilepis scolecina*.

1. After a careful examination, and after counting 10,000 of the nuclei in this region, I conclude that the growth of the sub-cuticula cannot be accounted for by mitotic or amitotic division.

2. Tissue growth is taking place rapidly in this region by the development of protoplasmic masses. My reasons for believing this are the following:

A. The nuclei in the multinucleate cells are frequently seen crowded together as if they had developed in protoplasmic masses.

B. In the protoplasmic masses the quantity of cytoplasm is out of proportion to the number of complete nuclei present.

C. Developing nuclei have been actually observed in the cytoplasm. The different stages of nuclear formation are shown by the following:

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[1] A similar process was suggested long ago by Schleiden and Schwann.

(*a*) The chromidia, or diffused nucleus.

(*b*) The irregular chromatin granules formed by the union of numerous chromidia and surrounded by a clear zone.

(*c*) The nuclear membranes of the nuclei in the masses vary considerably from delicate, scarcely discernible membranes to heavy, well developed ones.

D. These masses appear to arise by the simultaneous growth of cytoplasm and chromidial extrusions from the nuclei of certain cells.

3. The degenerate character of the nucleus is perhaps the result of the parasitic habit of the cestode.

I wish here to express my sincere thanks to Dr. R. T. Young, whose valuable criticisms and suggestions made this work possible. I also wish to express my indebtedness to Dr. B. H. Ransom for identifying my material.

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EXPLANATION OF PLATE.

FIG. 1. Multinucleate cell, *a*.

FIG. 2. Nuclei with indistinct membranes, *b* and *c*.

FIG. 3. Nuclei, *d* and *e*, lacking nuclear membranes.

FIG. 4. Chromatin granules, *f*, in the cytoplasm.

FIG. 5. Large chromatin granule, *g*, in cytoplasm.

FIG. 6. A developing protoplasmic mass, *h*, in which no definite nucleus is present.

FIG. 7. A lobe, *i*, of a protoplasmic mass in which no definite nucleus is present.

FIG. 8. A large protoplasmic mass in the body region which contains only one nucleus, *j*.

FIG. 9. Protoplasmic masses, *k*, developing in the body region.

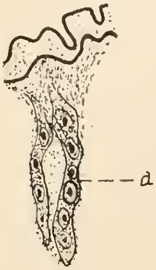


Fig. 1.

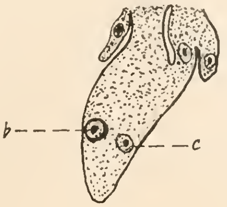


Fig. 2.

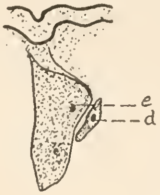


Fig. 3.

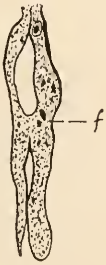


Fig. 4

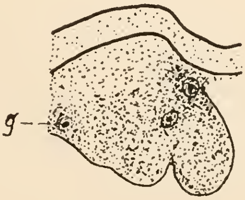


Fig. 5.

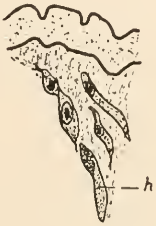


Fig. 6.

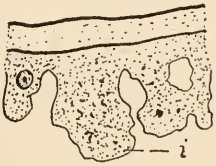
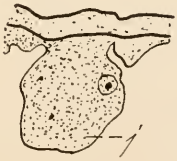


Fig. 7.



F.g. 8.

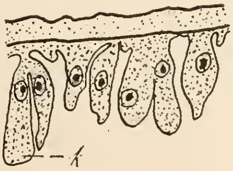


Fig. 9.

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